Electroconvulsive shock and 24-hour rhythms in the rodent brain

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ELECTROCONVULSIVE SHOCK AND 24-HOUR RHYTHMS IN THE RODENT BRAIN

Submitted by Anthony Lipovats

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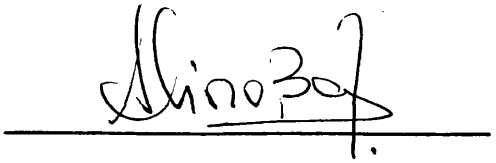

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CONTENTS

SUMMARY

The present study set out to examine the effects of electro-convulsive shock (ECS), in mice and rats, on behavioural and biochemical functions that are characterized by diurnal variation.

One series of experiments investigated the 24-hour fluctuation of tryptophan and 5-hydroxyindoleacetic acid concentrations in rat cerebrospinal fluid. Both substances were found to exhibit a significant variation over 24 hours, which did not appear to be affected by acute or chronic administration of ECS.

The circadian rhythm in locomotor activity was examined in mice and rats undergoing treatment with repeated ECS. The study was carried out under conditions of a normal light-dark cycle, constant light and constant dark. It was found that ECS did not induce consistent changes in the amount of activity of the animals or the frequency of the activity rhythm.

The passive avoidance response which also exhibited a diurnal variation, provided a model for evaluating learning and retention in mice. Repeated, but not acute, ECS was shown to cause both retrograde and anterograde amnesia.

Finally, a limited number of experiments involving the head-twitch response in the mouse indicated that repeated, but not acute, ECS possibly affects the postsynaptic function of the serotonergic system.

As a whole, the results indicate that chronic ECS does not affect the presynaptic regulation of 5-HT activity and that its mode of action might be more closely related with effects on 5-HT receptors.

The results also suggest that chronic ECS administration does

not interfere with the mechanisms responsible for the regulation or expression of diurnal rhythms.

The results are discussed in the light of the evidence that 5-HT is associated with the pathophysiology of depression, that circadian homeostasis may be abnormal during the illness and that electroconvulsive therapy, which is an effective antidepressant treatment, may do so by modifying the activity of the 5-HT system.

CHAPTER 1. PHYSIOLOGICAL ASPECTS OF THE 5-HT SYSTEM

1. PHYSIOLOGICAL ASPECTS OF THE 5-HT SYSTEM

1.1. Introduction

The objective of the first section of this introduction is to provide the reader with a concise report on the physiology and biochemistry of 5-hydroxytryptamine (serotonin, 5-HT), its precursor tryptophan and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the central nervous system (CNS). The concept of 5-HT binding sites will also be examined and associated with specific physiological roles for 5-HT and the action of various pharmacological agents.

1.2. Tryptophan Availability in the Body

Tryptophan is an essential amino acid occurring in the form of the L-enantiomer and obtainable from the diet under normal circumstances (Bender, 1982). Once it enters the circulation, tryptophan is unique among amino acids in that it is found in a bound form to plasma albumin with only a small portion (10-20%) being in a free state (Fernstrom, 1978; Green and Costain, 1979). About 98-99% of tryptophan is metabolised peripherally in the liver (Bender, 1982), where it enters the kynurenine pathway and is catalyzed by tryptophan pyrrolase. This enzyme is thought to be induced in the presence of its own substrate; corticosterone and hydrocortisone (cortisol) also increase tryptophan pyrrolase activity, leading to decreases in plasma tryptophan and, ultimately, brain tryptophan and 5-HT concentrations (Green, 1978; Green and Costain, 1979) and also 5-HIAA levels in whole brain and specific brain regions (Curzon and Green, 1971).

As can be seen in figure 1.1., the amount of tryptophan that is

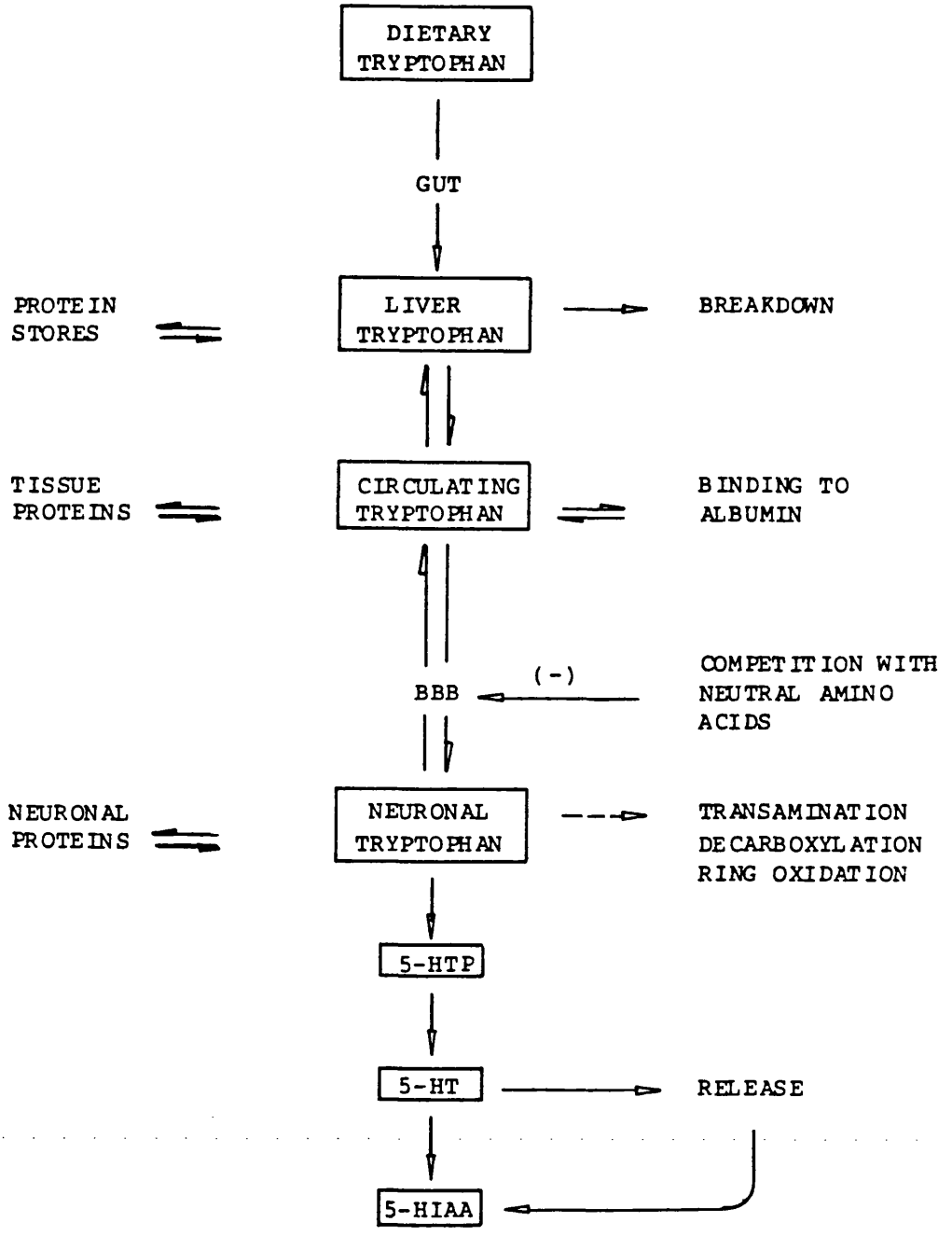


Fig. 1.1. The metabolic fate of dietary tryptophan in the body. BBB: Blood-brain barrier; 5-HTP: 5-hydroxytryptophan; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid.

available in the body through diet is distributed in many directions. A large part of it will be broken down or stored by the liver. The amount that enters the circulation will be partly used up to form proteins in the peripheral tissues whereas only a small part will cross to the brain, where it will be used for protein formation, but will also serve as a substrate for the synthesis of 5-HT (Fernstrom, 1978).

The rate of tryptophan uptake by the brain depends mainly on three factors: the amount of blood flow through a particular brain area, the concentration of tryptophan in blood and the activity of the transport system (Fernstrom, 1978). Since it is known that brain areas are supplied by varying amounts of blood (Bowman and Rand, 1980), it is reasonable to expect regional differences in the absorbed amounts of tryptophan.

The second variable that apparently has a crucial role in the movement of tryptophan in and out of the brain is tryptophan concentration in blood. It was mentioned above that tryptophan may be found in a free or albumin-bound form (Fernstrom, 1978). Although albumin binding seems specific, non-esterified fatty acids, which appear to have separate binding sites (Bender, 1982), displace tryptophan from albumin as, indeed, do various compounds such as salicylates, indomethacin and possibly phenothiazines (Bender, 1982). In view of these observations and also the influence of glucocorticoids on tryptophan availability it was important to specify the factors that affect tryptophan concentration in blood and its crossing to the brain. A question that has been raised and not conclusively answered so far, is whether free or total tryptophan controls the rate of tryptophan uptake by the brain. It has been

shown that administration of cortisol decreases the concentration of 5-HT and 5-HIAA both in whole brain and specific brain regions, indicating decreased 5-HT synthesis (Curzon and Green, 1971), an effect attributed to tryptophan pyrrolase induction by cortisol. However, the postulate that this enzyme may regulate plasma tryptophan has been challenged by the finding that both pyrrolase activity and plasma tryptophan concentrations reach their nadir at the same time of the day, namely between noon and early evening (Fernstrom, 1978), whereas one would expect the highest plasma tryptophan concentration during the period of decreased pyrrolase activity.

Similarly, it was noticed that brain and serum tryptophan concentrations, which normally vary during a 24-hour period, reached their highest levels at approximately the same time, i.e. during the night (Fernstrom, 1978). This suggested that brain tryptophan was dependent on serum tryptophan concentration, a notion strengthened by the finding that tryptophan administration caused an increase in both these variables (Fernstrom, 1978). This was taken as an indication that precursor availability may control 5-HT synthesis (Young and Gauthier, 1981). Also, food deprivation increased free plasma and brain tryptophan concentration by increasing the mobilization of non-esterified fatty acids (NEFA) in plasma and, consequently displacing tryptophan from albumin (Curzon, 1981). Indeed, a lot of data suggest that free, as opposed to total, tryptophan in plasma is the regulatory factor of brain tryptophan (Curzon, 1981; Knott and Curzon, 1972; Sarna, Kantamaneni and Curzon, 1985), since procedures that decrease or do not affect total

tryptophan, but increase the free portion, effectively increase brain tryptophan levels.

In contrast, it has been shown that: (a) insulin administration or carbohydrate ingestion elevate both serum total and brain tryptophan levels but decrease serum free tryptophan levels and (b) dietary increase of tryptophan was not accompanied by increments of either serum total or brain tryptophan (Fernstrom, 1978). These data clearly oppose the view on the significance of the free tryptophan fraction. A compromise is offered by the suggestion that, depending on circumstances, free tryptophan and a variable portion of bound tryptophan may control the brain content, since results in human CSF have shown a correlation of brain tryptophan with either free or total plasma tryptophan, depending on conditions (Young and Gauthier, 1981).

The third factor affecting the brain levels of tryptophan is its transport from blood to brain tissue. In order to appreciate the importance of this variable, it is necessary to consider in some detail the so-called blood-brain barrier.

1.3. Passage of Substances In and Out of the Brain

In general, there are two ways by which a substance can reach the brain: the principal one is via the blood supply and the other is via the cerebrospinal fluid.

1.3.1. Blood Supply to the Brain

The brain receives about 17% of the total cardiac output, although its weight in humans accounts for only 2% of the total, approximately (Barr, 1974). Despite the dense blood circulation,

which supplies the essential oxygen to the brain, the capillaries that transport blood do not allow a free passage of substances to and from the surrounding brain tissue. One reason for this is that the endothelium of the capillary lumen consists of particularly closely located cells, forming tight junctions, with less than 10% of capillaries being porous (Barr, 1974; Bowman and Rand, 1980; Pardridge and Choi, 1986). The second reason is that brain parenchymal astrocytic processes envelop the endothelial layer of the capillaries, offering an additional obstacle to the passage of substances (Barr, 1974). These two structural features are the anatomical basis of the blood-brain barrier (BBB), which is responsible for controlling the passage of substances in and out of the brain. However, the barrier is not purely a mechanical filter; among other factors, the lipid solubility, stereospecificity and protein binding of any given substance, the pH and osmotic tension gradients across the barrier also have determining roles (Bowman and Rand, 1980; Myers, 1975). It is noteworthy that in some brain areas, like the choroid plexuses, the area postrema in the brain stem, the pineal gland, the posterior pituitary gland and adjacent parts of the hypothalamus the BBB seems to be less effective (Barr, 1974; Pardridge and Choi, 1986).

1.3.2. The Cerebrospinal Fluid

The brain and spinal cord are supported and protected by a system of three membranes, the meninges. The thick outer meninge, the dura mater, is attached to the periosteum (Barr, 1974). The inner ones, the arachnoid and pia mater are separated by the subarachnoid

space which forms several expansions within the brain, the most important of which are the two lateral ventricles, the third and fourth ventricles and the cisterna magna. The subarachnoid space and the cisterns contain the cerebrospinal fluid (CSF) which thus bathes both the inside and outside surfaces of the brain and spinal cord. The CSF is produced mainly by the choroid plexuses of the lateral ventricles and eliminated through the arachnoid villi to the venous blood (Barr, 1974; Bowman and Rand, 1980). It is virtually free of cells and proteins, but contains glucose and electrolytes in quantities that vary from those in blood. Between the brain and CSF there exists a similar barrier, probably due to the same structural properties of the brain. Thus a substance injected directly into the CSF would not diffuse passively into the brain (Bowman and Rand, 1980). Equally, there is no free transportation between the vasculature of the pia mater and the CSF.

1.3.3. Transport Across the Blood-Brain Barrier

As a result of the above, the movement of various substances between the blood, brain and CSF is restricted. Specifically, between the brain and blood there are numerous identified active transport systems that facilitate and regulate the bidirectional movement of nutrients and drugs (Myers, 1975). So far, eight such independent systems have been identified and one of them is specific for neutral amino acids (Pardridge, 1986). Transport across the BBB through these systems appears to be the rate-limiting step for the neutral amino acids, because the affinity of the specific system for each amino acid is similar to the amino acid's plasma concentration; thus, concentrations of the competing amino acids

influence the uptake rate for each one of them (Hawkins, 1986). Since it has also been shown that the Km values for the BBB transport are low in rat brain and indirect evidence suggests that the same applies to the human brain, it has been suggested that availability of amino acids in the brain is dependent on their concentration in blood (Pardridge and Choi, 1986).

This data clearly implies that, apart from the absolute levels of free tryptophan in plasma, the ratio of tryptophan to the competing large amino acids (tyrosine, phenylalanine, leucine, isoleucine and valine) for the active transport system is at least equally important for the net influx of tryptophan to the brain as the factors previously alluded to. Still, it has been found that although plasma free tryptophan correlated with brain tryptophan after tryptophan infusion in humans, the concentration of competing amino acids was devoid of any regulatory effects (Gillman, Bartlett, Bridges, Hunt, Patel, Kantamaneni and Curzon, 1981).

1.4. Synthesis of 5-HT

The first step in the synthetic pathway of 5-HT in the brain involves the oxidation of tryptophan to 5-hydroxytryptophan (5-HTP) (Fig. 1.2), a reaction catalyzed by tryptophan hydroxylase (Fuller, 1985). This enzyme is believed to be specific for tryptophan in serotonergic neurons and is normally unsaturated with respect to its substrate (Fernstrom, 1978). It is this latter fact that allows the influence on 5-HT synthesis by manipulation of exogenous tryptophan; were it not unsaturated, the "window" for changes due to precursor availability would be very narrow and plasma and brain tryptophan levels would not affect 5-HT formation significantly. Thus,

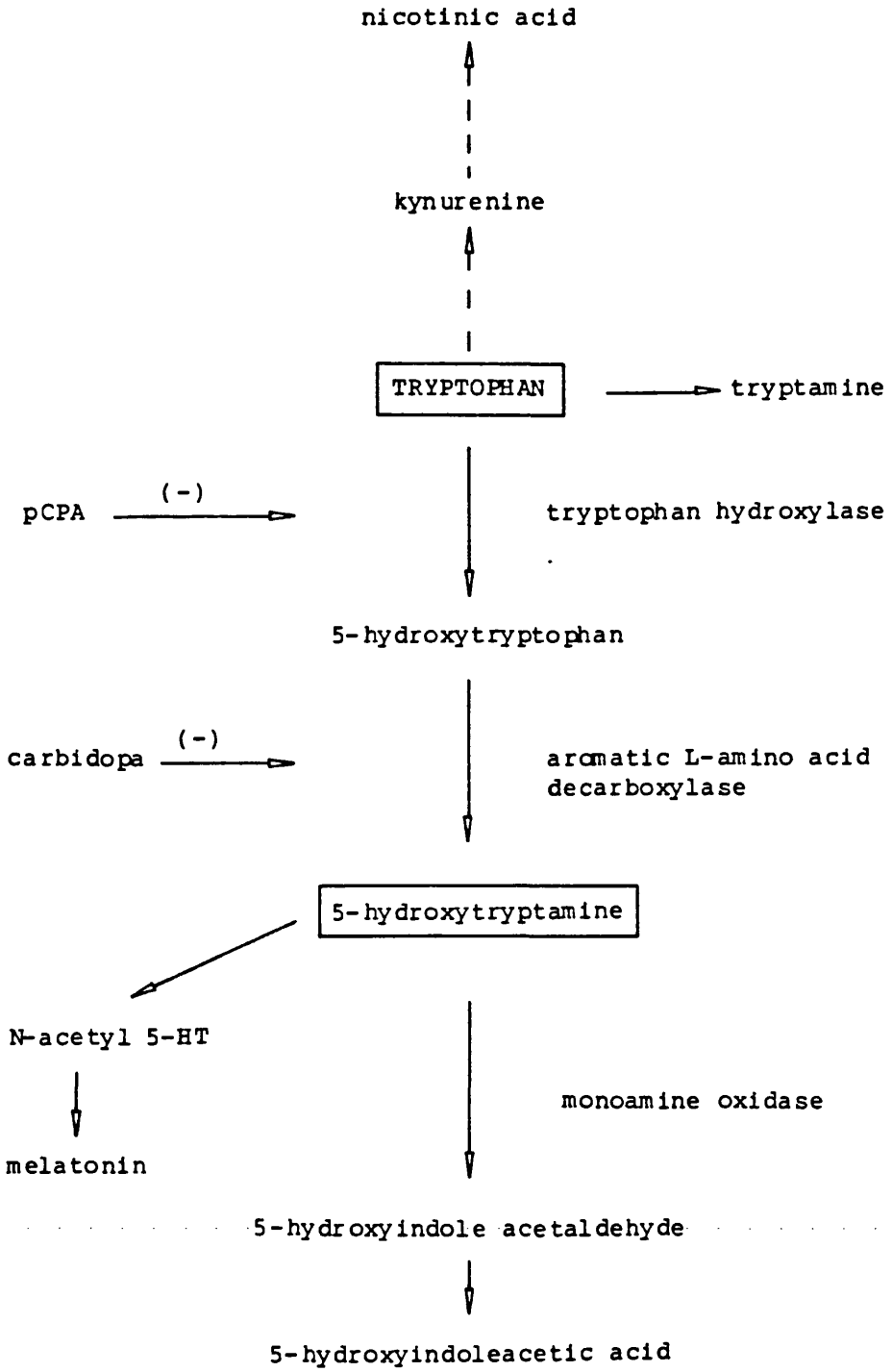


Fig. 1.2. The main metabolic pathways for tryptophan

(Adapted from: Airaksinen and Airaksinen, 1978)

tryptophan hydroxylase is considered the rate-limiting step in 5-HT synthesis (Bender, 1982). The enzyme requires molecular oxygen, ferrous ions and a reduced pteridine cofactor, tetrahydrobiopterin (Fernstrom, 1978). The intermediate product, 5-HTP, is rapidly decarboxylated to 5-HT and thus is only found in very low concentrations in the brain. The oxidation to 5-HT is mediated by aromatic L-amino acid decarboxylase (AAAD), an enzyme non-specific to 5-HT (Fuller, 1985) since it also mediates the formation of dopamine from L-dopa. This enzyme requires pyridoxal phosphate as a cofactor (Fernstrom, 1978) and its Km is remarkably higher than that for tryptophan hydroxylase.

The newly synthesized 5-HT may be either stored in cytoplasmic vesicles or metabolized intraneuronally.

1.5. Metabolism of 5-HT. Monoamine Oxidase

Metabolism of 5-HT before or after release, takes place in two steps: first, 5-HT is oxidised by monoamine oxidase (MAO) to 5-hydroxyindole acetaldehyde which is further converted by aldehyde dehydrogenase to yield 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of 5-HT in CNS (Fernstrom, 1978; Fuller, 1985; Green, 1978). The enzyme MAO is a flavoprotein, widely distributed in the brain, located intraneuronally on the outer mitochondrial membrane, but also found extraneuronally (Fagervall and Ross, 1986; Green, 1978; Youdim and Finberg, 1983).

The enzyme exists in at least two forms, types A and B (Youdim and Finberg, 1983). The two types differ in their substrate specificity, antagonist sensitivity and location within the brain but it is still unknown whether these differences indicate two separate

enzyme entities, two active sites of the same enzyme or two locations on the mitochondria (Youdim and Finberg, 1983).

With respect to substrate specificity the table below shows which type is associated with the oxidation of which substrate:

TYPE A	TYPE B	TYPES A + B
Noradrenaline	Phenylethylamine	Dopamine
Adrenaline	Benzylamine	Tyramine
5-HT		

(Fuller, 1985; Wolf, Youdim and Kuhn, 1985; Youdim and Finberg, 1983).

It should be noted that specificity varies between species and is only relative so that when, for example, MAO-A is inactivated, MAO-B can take over the metabolism of 5-HT (Fuller, 1985; Ross, 1986).

The substances that inhibit MAO usually do so irreversibly and are not highly selective. Clinically or experimentally useful MAO inhibitors (MAOIs) include:

clorgyline (irreversible MAO-A selective)

deprenyl and pargyline (irreversible MAO-B selective)

tranylcypramine, phenelzine, isocarboxazide and iproniazid (irrevesible non-selective)

(Fuller, 1985; Youdim and Finberg, 1983).

Administration of these drugs results in accumulation of 5-HT and decrease in 5-HIAA concentration. Interestingly, there are indications that up to 80% of MAO has to be inhibited before any behavioural or therapeutic effects are manifested (Pare, 1985). If one is to accept that 5-HT deficiency has a causative role in depression (see section 2.4.2), it follows that specific MAO-A

inhibitors would be essential. Reversible, MAO-A-specific inhibitors like harmaline and (+)-amphetamine have not found clinical use but newer agents, like toloxatone (Pohl and Gershon, 1983) or amiflamine (Fuller, 1985) among others, might lead to more positive results.

1.6. Release and Neuronal Activity of 5-HT

The vesicular 5-HT is available for release mainly by exocytosis (Wolf, Youdim and Kuhn, 1985) and there is evidence that newly synthesized amounts of 5-HT are preferentially released (Elks, Youngblood and Kizer, 1979b).

So far, the presented evidence mainly indicates that tryptophan availability affects brain tryptophan concentrations. There is, however, no definite indication as to whether increased brain tryptophan results in increased 5-HT synthesis. Certainly, since a rise in brain tryptophan levels correlated with increased levels of CSF 5-HIAA, it was assumed that 5-HT synthesis does increase after tryptophan loading (Gillman et al, 1981; Lasley and Thurmond, 1985; Lookingland, Shannon, Chapin and Moore, 1986; Young and Gauthier, 1981). On the other hand, studies in brain slices disclosed that tryptophan concentration and uptake do not affect brain synthesis (Elks et al, 1979b).

Moreover elevated tryptophan concentration does not seem to affect release of tryptophan from its storage pools (Elks et al, 1979b; Lookingland et al, 1986). It is useful to remember that tryptophan uptake by brain neurons is not specific and thus dopaminergic neurons may also be responsible for tryptophan uptake (Elks et al, 1979b) and possibly even transformation to 5-HT (van

Praag, Flentge, Korf, Dols and Schut, 1973), although the latter seems improbable in view of the discrete localization of tryptophan hydroxylase.

Finally, the question arises whether precursor availability influences 5-HT function. The putative hypnotic and antidepressant actions of tryptophan (Young and Gauthier, 1981) would suggest that, indeed, tryptophan administration somehow affects 5-HT activity. Tryptophan infusions have been found to cause sedation and impairment in performance tests (Winokur, Lindberg, Lucki, Phillips and Amsterdam, 1986), whilst it has also been shown that the latency to optical stimulation of cortical potentials correlated with ventricular 5-HIAA concentrations (Curzon, 1981).

In contrast, little justification for similar assumptions was provided by the results in hypothalamic tissue, following administration of tryptophan (Lookingland et al, 1986) and electrophysiological studies in cat raphe neurons, following dietary manipulations (Trulson, 1985).

1.7. The Significance of 5-HIAA in CSF

After release, 5-HT may activate the specific 5-HT receptors before it is taken up by the presynaptic neuron, so that it is eliminated from the synaptic cleft and oxidized to 5-HIAA.

Since 5-HIAA is the major metabolite of neuronal 5-HT, a lot of research has been devoted to establishing how good an index of 5-HT activity it is. Moreover, its identification in CSF initiated the attractive hypothesis that it might prove a useful marker of psychiatric and neurological disorders as well as an index of therapeutic outcome. However, there are several problems which

diminish the usefulness of CSF: it is not known what proportion of brain 5-HIAA enters the CSF and what proportion is excreted directly into the bloodstream. Also, since CSF is almost invariably sampled from the lumbar region in humans, it is debatable to what extent lumbar 5-HIAA is of brain origin.

In a study of the kinetics of 5-HIAA excretion from rat brain, using both experimental animals and computer simulation (Burns, London, Brunswick, Pring, Garfinkel, Rabinowitz and Mendels, 1976) the following observations were made. First, in the rat, the amount of 5-HIAA transported directly from the brain to the circulation in negligible. Consequently, brain 5-HIAA comes in rapid equilibrium with ventricular 5-HIAA (fig. 1.3). Also there is a rapid transport of 5-HIAA from the ventricles to the cisterna magna and a gradient between the two areas which disappears when probenecid is administered (Fig. 1.3).

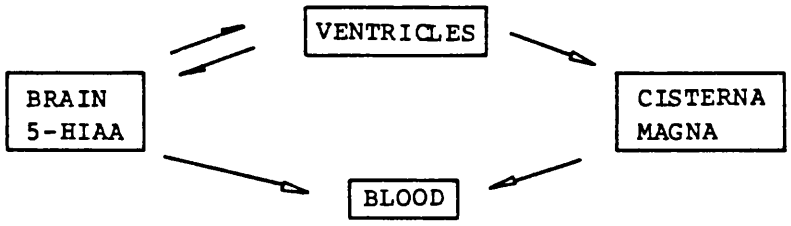


Fig. 1.3

In direct contrast, another study showed that possibly up to 90% of 5-HIAA may leave the brain by direct excretion to the bloodstream, thus bypassing the CSF (Meek and Neff 1973).

Also there is conflict as to whether 5-HIAA levels reflect 5-HT activity. Thus there is evidence that brain 5-HIAA is an index of intraneuronal metabolism of 5-HT, rather than released 5-HT

(Lookingland et al, 1986) and, as such, a measure of MAO activity (Wolf, Youdim and Kuhn, 1985). Predictably, there is also sufficient data for other authors to claim that 5-HIAA concentration in brain does reflect neuronal activity (Shannon, Gunnet and Moore, 1986).

The interest in CSF levels of 5-HIAA was stimulated by the use of probenecid which blocks the active transport of 5-HIAA and also homovanillic acid (HVA), the principal dopamine metabolite, from CSF to blood. Probenecid administration thus leads to accumulation of these two acidic metabolites, from which accumulation turnover rates for 5-HT and dopamine can be calculated (van Praag, 1981). The method is not without its hazards. It is impossible to ascertain whether the blockage is complete and, on top of this, it is known that, to a certain extent, probenecid can mobilize tryptophan from albumin and thus increase free tryptophan with the expected increase in brain tryptophan concentrations (Perel, Levitt and Dunner, 1974; van Praag, 1981). Such an increase in plasma free tryptophan has been obtained, at least after repeated probenecid administration, and correlated with the plasma levels of probenecid (Lewander and Sjostrom, 1973).

However, in subsequent studies the doubts deriving from the effect of probenecid on brain tryptophan metabolism in humans were minimised (van Praag, Korf and Schut, 1973); the authors concluded that any changes in 5-HIAA (and HVA) accumulation seen in CSF after probenecid pretreatment are a direct reflection of 5-HT (and dopamine) turnover and not a result of probenecid uptake or effects on 5-HT (and dopamine) synthesis. Lack of effect of probenecid on CSF tryptophan concentration was also reported for the rat and showed that, overall, 5-HIAA levels in cisternal CSF may lead to a

reasonable estimation of brain 5-HIAA concentration and 5-HT turnover (Sarna, Hutson, Tricklebank and Curzon, 1983).

Finally, the question of the origin of lumbar 5-HIAA is still open. A number of studies in animals and man with partial block of CSF flow or spinal cord transection have led to opposing results and the general conclusion that lumbar 5-HIAA is, to some extent, a reflection of brain 5-HT turnover, but with variable contributions from spinal 5-HT metabolism (Abrams, 1978; Curzon, 1978). Similar data has disclosed that HVA in CSF is of exclusively brain origin, whereas 3-methoxy-4-hydroxyphenylglycol (MHPG), the primary metabolite of noradrenaline in CNS, shows no concentration gradient between ventricular and lumbar CSF, a sign that the spinal cord contributes significantly to its CSF concentration (Garelis, Young, Lal and Sourkes, 1974).

1.8. Receptors for 5-HT

1.8.1. Introduction

Ever since the discovery that serotonin acts on specific receptors, a body of evidence has accumulated in an effort to link specific effects to binding sites. The identification of radioligand binding sites for 5-HT in the CNS triggered a spectacular research effort which resulted not only in a signficant increase in our knowledge about the regulation of the 5-HT system and its physiological correlates but also to a good deal of controversy as to the accuracy and specificity of most of these findings. Below, the current status of 5-HT binding sites will be briefly reviewed before any attempt to connect these sites with particular physiological responses.

The crucial step in the identification of binding sites is the clear demonstration of specific ligands for any putative site. With the aid of labelled 5-HT and spiperone, it was initially found that part of the population of binding sites, as defined by binding of labelled lysergic acid diethylamide (LSD) could be discriminated as 5-HT_1 binding sites with high affinity for 5-HT whereas another subpopulation showed high affinity for spiroperidol and was called 5-HT_2 (Peroutka and Snyder, 1979).

1.8.2. 5-HT_1 Binding Sites

Since their initial identification by ^{3}H-5HT, research has focused on providing more specific ligands. Metergoline, metitepine and LSD have all been tried but their use is hampered by their high non-specific binding (Leysen, 1985). The search for a specific antagonist has not met with success so far. Compounds like methysergide and the β-adrenergic receptor antagonists (-)propranolol, pindolol and cyanopindolol have been used but possess significant non-specific binding properties (Bradley, Engel, Feniuk, Fozard, Humphrey, Middlemiss, Mylecharane, Richardson and Saxena, 1986; Leysen, 1985). A noticeable characteristic of 5-HT_1 binding sites is their resistance to putative 5-HT_2-selective antagonists like ketanserin (Bradley et al, 1986).

1.8.2.1. The 5-HT_{1A} Binding Site

From results with spiperone, a compound that acts on 5-HT as well as dopamine binding sites, it was shown that the 5HT_1 population could be distinguished in two subpopulations, one with high affinity

($5\text{-}HT_{1A}$) and one with low affinity ($5\text{-}HT_{1B}$) (Hamon, Cossery, Spampinato and Gozlan, 1986; Tricklebank, 1985).

The $5\text{-}HT_{1A}$ binding site has also been identified by the agonist 8-OH-DPAT. The compound is believed to stimulate adenylate cyclase, thought to be linked to $5\text{-}HT_1$ binding sites, although this is a highly disputed attribute (Leysen, 1985). The putative effect is blocked by the non-specific antagonist spiperone but not by the $5\text{-}HT_2$-selective antagonist ketanserin (Middlemiss, 1986). Other agents with higher selectivity that have been used to describe the $5\text{-}HT_{1A}$ site include buspirone and isapirone (TVX Q 7821).

It has also been shown that 8-OH-DPAT seems to have only slight effect at the terminal autoreceptor which is identified by cyanopindolol and more for the cell body autoreceptor which is assumed to be of the $5\text{-}HT_{1A}$ type (Middlemiss, 1986).

1.8.2.2. The $5\text{-}HT_{1B}$ Binding Site

There are no compounds that can characterize conclusively the $5\text{-}HT_{1B}$ binding site. Quipazine, cyanopindolol and some piperazine derivatives have been used to label the site but their specificity is only marginally higher than that for other sites ($5\text{-}HT_{1A}$ or β-adrenergic receptors). Consequently, the presence of $5\text{-}HT_{1B}$ receptors can only be calculated by deduction of the other sites from the total bound population (Hamon et al, 1986). By this method, whereas $5HT_{1A}$ sites are thought to dominate the $5\text{-}HT_1$ population in the hippocampus, it appears that $5\text{-}HT_{1B}$ sites abound in striatum (Hamon et al, 1986).

Recent studies have shown that RU 24969, a $5\text{-}HT_1$-selective

agonist may show more selectivity for the 5-HT_{1B} site (Frances and Simon, 1986; De Souza, Goodwin, Green and Heal, 1986).

1.8.2.3. The 5-HT_{1C} Binding Site

Hardly anything is known about these sites; they account for only a small percentage of the total 5-HT_1 binding site number and are possibly denser in the choroid plexus (Hamon et al, 1986).

1.8.3. 5-HT_2 Binding Sites

The identification of 5-HT_2 binding sites was initially achieved by demonstrating that they constituted a population with low affinity for 5-HT and high affinity for spiroperidol and also methysergide, methiothepin and cyproheptadine (Bradley et al, 1986; Leysen, 1985; Peroutka and Snyder, 1979). Another antagonist which has been shown to have a great affinity for 5-HT_2 sites is ketanserin; this compound is, to date, the most appropriate ligand for the characterization of 5-HT_2 binding sites despite its considerable affinity for histamine H_1 and α-adrenergic receptors, since it has a very low affinity for 5-HT_1 sites (Bradley et al, 1986; Leysen, 1985). Unfortunately, so far no suitable agonists have been described for 5-HT_2 sites (Bradley et al, 1986). Finally, 5-HT_2 sites have been identified in frontal cortex, nucleus accumbens and olfactory tubercle (Leysen, 1985).

1.8.4. 5-HT_3 Binding Sites

Since 5-HT_3 binding sites have, so far, been identified and correlated with specific functions mostly in the periphery (Bradley et

al, 1986), they will not be examined here.

1.8.5. 5-HT Autoreceptors

There is considerable evidence suggesting that 5-HT neurons are modulated, at least partly, by a negative feedback system through autoreceptors (Neff and Neckers, 1981; Moret, 1985; Verbeuren, Coen, Schoups, Van de Velde, Baeyens and De Potter, 1984). These autoreceptors are located on 5-HT neurons in the raphe nuclei and inhibit the spontaneous firing whereas other autoreceptors are located presynaptically on 5-HT nerve terminals and inhibit the stimulation-induced release of 5-HT (Moret, 1985). It is believed that both kinds of autoreceptor bear similarities to the 5-HT_1 binding site (Moret, 1985) and possibly the presynaptic autoreceptors are of the 5-HT_{1B} subtype, whereas the ones on the cell bodies are of the 5-HT_{1A} subtype (Middlemiss, 1986). Serotonin and agonists like LSD and ergocornine activate the autoreceptor to produce inhibition of firing and release of 5-HT whilst antagonists like methiothepin block this inhibitory effect (Moret, 1985). The data provided by these studies strongly suggest that there is a functional role for 5-HT autoreceptors but more work is needed to support such a claim.

1.9. Localisation of 5-HT Neurons

Using a variety of techniques the distribution of 5-HT neurons has been extensively studied, especially in the rat. It is now known that the majority of cell bodies are found in the raphe nuclei. From there, projections innervate the pons, medulla and cerebellum. Ascending pathways cross the medial forebrain bundle and project into many parts of the limbic system, the hypothalamus, thalamus and

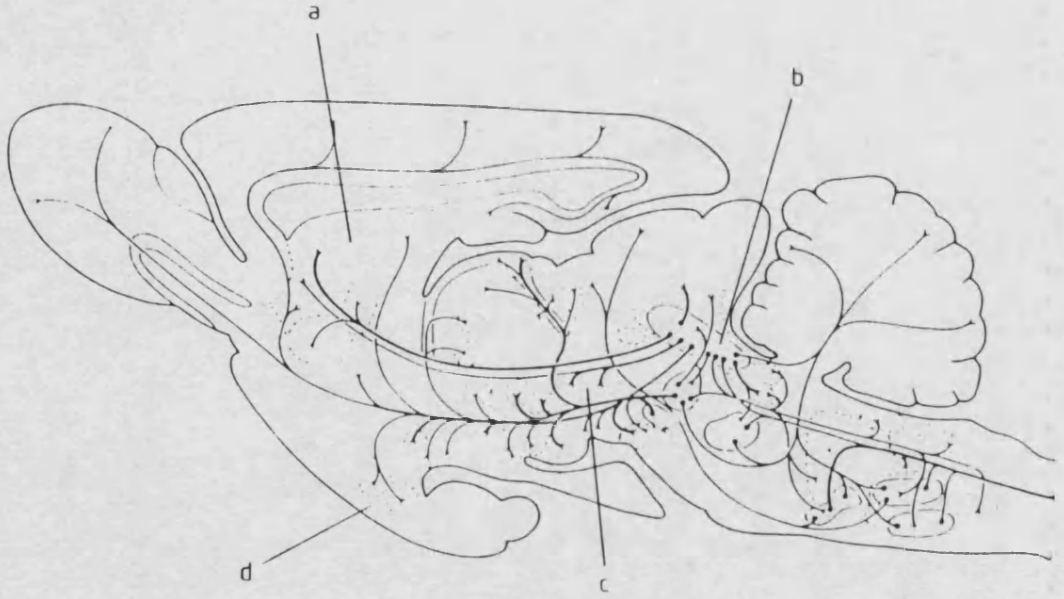


Figure 1.4 Schematic diagram of the serotonergic system of the rat brain with ascending and descending pathways from the raphe nuclei. (a) nucleus caudatus putamen, (b) nucleus raphe dorsalis, (c) substantia nigra, (c) nucleus amygdaloideus.

(Adapted from: Iversen, 1984)

cerebral cortex (Fig. 1.4.). Also, from the medulla oblongata tracts end up in the grey matter of the spinal cord whereas another important pathway crosses from the hypothalamus to the pituitary gland (O'Brien, 1978).

Nerve endings are essentially ubiquitous but some areas like the brainstem and hypothalamus are particularly rich in 5-HT terminals (O'Brien, 1978).

1.10. Physiological Roles for 5-HT

There are many physiological functions and behaviours in which 5-HT has been implicated.

The existing evidence suggests that 5-HT is involved in the regulation of temperature, since elevation of 5-HT concentration induces hyperthermia (Green, 1978). The possible mechanisms mediating this effect are discussed in section 1.11.3.

Also, 5-HT seems to play an important part in learning and the formation of memory (section 5.5.1.2).

Sexual behaviour appears to be enhanced by procedures that decrease 5-HT concentration in brain (Green, 1978), whilst the same procedures increase pain sensitivity (Green, 1978). Conversely 5-HT enhancement potentiates morphine analgesia (Sparkes and Spencer, 1969).

Depletion of 5-HT causes insomnia and administration of L-tryptophan is believed to induce sleep (Green, 1978).

There is also a lot of evidence connecting the 5-HT system with many behavioural aspects and these are reviewed in section 1.11., in conjunction with the description of 5-HT receptors.

Finally, there is a potentially crucial role for 5-HT in the regulation of mood and the pathology of depression. This aspect is extensively analysed in Chapter 2.

It should be borne in mind that none of these functions is solely attributable to 5-HT. Besides inputs from the periphery, other established neurotransmitter or neuropeptide systems are involved in the regulation of functions like the ones described above, so that the end result should be conceived as a sum of influences of the various systems.

1.11. Functional Correlates of 5-HT Receptors

In the preceding sections, various drugs were already labelled as agonists or antagonists. Such an attribute requires primarily the demonstration of a specific active site for a drug and a consequent specific and measurable function or behaviour. This section is set out to demonstrate a few of the correlations between certain responses and specific receptors and drugs.

1.11.1. The 5-HT Syndrome

Drugs that are known to increase 5-HT availability in the neuron induce the so-called 5-HT syndrome in the rat, which is characterised by the following behavioural changes: reciprocal forepaw treading, head-weaving, trunk weaving, flat body posture, hind limb abduction, Straub tail, body tremor, piloerection, hyperlocomotion, hyperreactivity and other, less readily quantifiable changes (Green, 1984; Tricklebank, 1985).

There are three ways by which the syndrome can be elicited: (a) procedures that increase the synthesis or decrease the degradation of

5-HT, such as: high doses of 5-HTP, low doses of 5-HTP plus a MAOI, L-tryptophan plus a MAOI, low doses of 5-HTP plus a 5-HT uptake inhibitor or a peripheral decarboxylase inhibitor. (b) drugs which release 5-HT, like p-chloroamphetamine (PCA) and fenfluramine, and (c) direct receptor agonists like 5-methoxy-dimethyltryptamine (5-MeODMT), 5-methoxytryptamine (5-MeOT), LSD, mescaline and also quipazine and other piperazines (Green, 1984). The site of initiation of the syndrome has been identified as the hindbrain and spinal cord and a common criticism is that binding studies are performed at locations like the frontal cortex without having established any working relation between the initiation site and the postsynaptic sites in the description of a certain function (Green and Heal,1985).

The administration of L-tryptophan does not induce a syndrome unless the activity of MAO is blocked,in which case the increase in 5-HT synthesis is in excess of its elimination (Green, 1978). The syndrome can also be elicited in reserpinized rats treated with PCA (Kuhn, Wolf and Youdim, 1985). When 5-HT synthesis is inhibited by p-chlorophenylalanine (pCPA), the syndrome is not elicited by procedures which rely on the production or availability of 5-HT but is obtainable after direct receptor stimulation (Grahame-Smith, 1971; Green, 1984).

There is evidence that the appearance of the syndrome is not solely dependent on the 5-HT system, but that the noradrenergic and dopaminergic systems also contribute. Salbutamol and clenbuterol enhance whereas propranolol antagonizes the behaviour (Cowen, Grahame-Smith, Green and Heal, 1982; Green, 1984). The effect is apparently attributable to direct postsynaptic response in view of

the action of β-adrenergic receptor antagonists at 5-HT receptors and the fact that depletion of noradrenaline by disulfiram or 6-hydroxy-dopamine (6-OHDA) lesioning were without effect (Green, 1984).

Moreover, α-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor, abolishes the syndrome even if it is caused by 5-MeODMT, whereas L-dopa can restore it (Green, 1978). Hyperactivity and hyperreactivity are the two behavioural changes that are thought to possess a dopaminergic link (Green, 1984). This link may receive both positive and negative signals from activation of 5-HT_1 and 5-HT_2 receptors respectively (Green, Guy and Gardner, 1984).

Although in general 5-HT antagonists inhibit the appearance of the syndrome, it has been noted that methysergide and metergoline potentiated hyperlocomotion and hyperreactivity (Green, Hall and Rees, 1981; Stolz and Marsden, 1981; 1982). Morever, cyproheptadine, mianserin and cinanserin only inhibited the syndrome when it was caused by quipazine but not 5-MeODMT (Green et al, 1981; Green, 1984).

These are only a few of the signs that the precipitation of the syndrome is not a matter of activation of one receptor subtype or of one neurotransmitter system. Already, the increase in locomotor activity and reactivity have been partly related to the action of dopamine. Head-weaving, hind limb abduction and forepaw treading are inhibited by the 5-HT_2 selective antagonists ketanserin and pirenperone and also metergoline and methysergide. These behaviours are prominent after administration of the putative 5-HT_{1A} agonist 8-OH-DPAT and antagonized, by and large, by most 5-HT_1, 5HT_2, dopamine or α_1-adrenergic antagonists (Green, 1984). Ketanserin, for example, inhibited the response but only at low doses (Goodwin and Green,

1985).

Hyperlocomotion and hyperreactivity were observed when the syndrome was produced by 5-MeODMT, and despite the presence of pirenperone which blocked all the other behaviours. Also, they were present after administration of the 5-HT_1 agonist RU 24969 (Green, 1984; Green et al, 1984). These data, along with other evidence, indicated that hyperlocomotion and hyperreactivity may be attributable to activation of 5-HT_{1B} receptors, whereas the remaining behavioural aspects of the syndrome are mediated by 5-HT_{1A} and 5-HT_2 receptors (Green, 1984).

1.11.2. The Head-Twitch Response

If the same pharmacological increases of 5-HT function (5-HTP, alone or with carbidopa; 5-MeODMT; LSD; quipazine) are conducted on mice, the prevalent symptoms are increased locomotor activity and a distinct head-twitch response, which corresponds to the "wet-dog shake" in the rat; both behaviours are blocked by 5-HT antagonists (Green, 1984). As was the case for the 5-HT syndrome, β_2-adrenergic agonists enhanced the head-twitch response, although β-adrenergic antagonist did not appear to block it (Goodwin and Green, 1985; Green, 1984). Interestingly, α_2-adrenergic agonists (clonidine, guanabenz) inhibited the response whereas α_2-antagonists yohimbine and piperoxane enhanced it (Green, 1984).

Since there are no known 5-HT_2 specific agonists, 8-OH-DPAT and RU 24969 were used but failed to induce the head-twitch response (Goodwin and Green, 1985; Green, 1984). Ketanserin, pirenperone and ritanserin, all potent 5-HT_2 antagonists have been shown to

antagonize the response (Green, 1984). Moreover, procedures that decreased the number of 5-HT_2 receptors, like chronic administration of mianserin or desmethylimipramine (DMI), inhibited the response whereas electroconvulsive shock (ECS) increased the 5-HT_2 receptor number and enhanced the response, thus suggesting that the head-twitch behaviour in mice is 5-HT_2-receptor mediated (Goodwin, Green and Johnson, 1984; Green, 1984). The "wet-dog shake" behaviour in the rat has also been shown to relate more to 5-HT_2 function (Lucki, Nobler and Frazer, 1984).

1.11.3. Temperature Regulation

The administration of L-tryptophan and tranylcypromine in rats produced hyperpyrexia as also did 5-MeODMT, whose action was potentiated by the MAOI (Grahame-Smith, 1971). This early finding suggested that there might be a 5-HT receptor-mediated effect on thermoregulation.

A hyperthermic response can be elicited by the 5-HT agonists MK 212, quipazine and high doses of 5-MEODMT (Gudelsky, Koenig and Meltzer, 1986; Goodwin and Green, 1985). Hypothermia can be induced by the 5-HT_{1A} agonist 8-OH-DPAT, low doses of 5-MeODMT, the antagonists ketanserin and pirenperone and also clonidine and apomorphine (Goodwin and Green, 1985; Goodwin et al, 1985; Green et al, 1984; Gudelsky et al, 1986). The interpretation of these data pointed towards a role for the 5-HT_{1A} receptor in temperature regulation.

The 8-OH-DPAT-induced hypothermia is a dose-dependent response, antagonized by spiperone, methiothepin and pindolol, all of which have high affinity for the 5-HT_{1A} binding site (Gudelsky et al,

1986). Following chronic administration, 8-OH-DPAT caused an attenuated response to hypothermic doses of 8-OH-DPAT, as did also the putative 5-HT_1 presynaptic antagonist isapirone (De Souza et al, 1986). The putative 5-HT_{1B} agonist RU 24969 failed either to affect temperature or to alter 8-OH-DPAT-induced hypothermia (Green et al, 1984; De Souza et al, 1986).

As a conclusion, it seems that activation of 5-HT_2 receptors raises body temperature whereas 5-HT_{1A} receptors precipitate the opposite result and 5-HT_{1B} receptors are not involved in thermoregulation (Green, 1984).

1.12. Conclusions

If the above discussion has succeeded in producing a clear-cut picture of the factors that affect various physiological functions or drug-induced behaviours, it may have failed to demonstrate that the data are merely scattered pieces of a complex network that involves not only bimodal regulation from the same system, but also numerous inputs from the dopaminergic, adrenergic and cholinergic systems, to mention but a few (Green, 1984; Handley and Singh, 1986; Tricklebank, 1985). Thus, any attempts to attribute specific functions to a receptor subtype or even a neurotransmitter system should be viewed with caution. Conversely, drug effects rarely reveal the true mediator of a response, since they frequently lack the selectivity and specificity that would allow a unique intepretation of the biochemical events that elicit the response.

These arguments and also the problems in interpreting the significance of 5-HIAA in the CSF should be borne in mind when, in

the next chapter, the role of 5-HT in, and the pharmacological treatments of, depression will be examined.

CHAPTER 2. AFFECTIVE DISORDERS-DEPRESSION

2. AFFECTIVE DISORDERS-DEPRESSION

2.1. Introduction

The concept of affective disorders as a clinical entity is primarily based on the consistent finding of mood alterations. The broad distinction into depression and mania is made in terms of their relation to mood lowering and elevation respectively, although many other symptoms are now considered at least equally important in the description of the disorder, particularly the milder forms (Burrows and Davies, 1983; Hamilton, 1979a).

This section will focus primarily on depression but mania will also be considered, since it is closely connected with certain types of depression. A brief review of the major symptomatology and classification criteria will be followed by an analysis of the biochemical findings in depressive patients. Finally, pharmacological and non-pharmacological treatments will be discussed in relation to the biochemical and clinical findings.

2.2. Characteristics and Classification

The broad group of affective disorders consists of depressive illness, manic-depressive disorder and anxiety neurosis (Hamilton, 1979a).

The onset and time-course differ considerably between depressive and manic-depressive illness and are related to age, sex and socio-economic factors (Hamilton, 1979a). A common finding is that, if followed up for sufficient lengths of time, almost all patients show cycles of illness and recovery. The length of each episode, the latency to the next one and their number may be related to the nature of the illness, the age of onset and the chronicity of the illness,

among other factors (Angst, 1981; Hamilton, 1979a). Finally, there is a high incidence of affective illness among the relatives of diagnosed patients (Hamilton, 1979a) but a genetic marker has, so far, not been found.

Many systems have been described which attempt to classify depressive patients in a way that would allow the medical world to draw conclusions from the comparisons of the groups in terms of treatment response, prognosis, course of the illness and hereditary importance. The most important distinction, by far, is that between bipolar and unipolar depression based on the course of the illness; the former is characterized by depressive episodes alternating with manic episodes whereas the latter manifests itself as depressive episodes interrupted by, frequently complete, remission (Hamilton, 1979a).

Furthermore, depression can be characterized as endogenous or reactive (depending on whether a constitutional or an exogenous cause is suspected); primary or secondary (when there is an absence or presence of a history of previous non-affective psychiatric illness); and psychotic or neurotic (depending on the nature and severity of the symptoms (Gelder, Gath and Mayou, 1983; Hamilton, 1979a; Klerman, 1978; Rees, 1982). These names by nature introduce aetiological and symptomatic considerations which are not always accurate. A third kind of depression is possibly involutional depression or involutional melancholia although it is not accepted generally as a separate syndrome (Gelder et al, 1983; Hamilton, 1979a).

2.3. Symptoms of Depression and Mania

Depression should not be confused with depressed mood that anyone may show under adverse circumstances like grief and which is a normal, transient reaction. Persistence, on the other hand, is often what urges the first contact with a doctor.

2.3.1. Depression

In the mild stages of the illness, the patient usually complains of lack of energy, difficulties with sleep, low mood, which frequently fluctuates during the day, and loss of interest, appetite and libido. As the condition progresses, patients typically display a tendency to wake up early in the morning, have interrupted sleep, difficulties in falling asleep and unpleasant dreams. Weight loss and bodily pains are also encountered. He/she also feels isolated, self-reproachful, shows impaired judgement, excessive or inappropriate guilt and emotional instability. All these build up an attitude for the past, present and future that may lead to preoccupation with thoughts of death, suicidal tendencies and attempts. The physical appearance of the patient can often tell much about the condition itself. Retardation and agitation may alternate or coexist but one of them tends to dominate, in severe forms. Physical symptoms of anxiety, like sweating, palpitations and trembling, and slowed speech and delayed responses are, finally, a few more often presented symptoms (Concensus Development Panel, 1985; Burrows and Davies, 1983; Gelder et al, 1983; Hamilton, 1979a; Rees, 1982).

2.3.2. Mania

The initial stages of the illness are increased activity, optimism and cheerfulness. The patient talks continually, becomes excessively friendly, sexually and socially uninhibited and tactless, and occupies himself/herself with many different tasks. In terms of work, he/she makes overestimated judgements, takes decisions that cannot be met practically or financially, and cannot tolerate criticism or advice from the familial or social environment. Increased appetite, libido and decreased sleep requirements round up the image. Eventually, these symptoms reach a point of ceaseless activity and talking, excessive euphoria and even rage. Hallucinations and delusions may also be present (Consensus Development Panel, 1985; Hamilton, 1979a; Rees, 1982).

2.3.3. Rating Scales

The assessment and evaluation of the described symptoms can be standardized by the use of rating scales. These scales may be used by the interviewer-therapist (observer scale), the most common one being the Hamilton Rating Scale, or by the patients (self-assessment scales), examples of which are the Beck Depression Inventory and the Zung Self-Assessment Depression Scale (Hamilton, 1979b). The classification into diagnostic categories is aided by the International Classification of Diseases (ICD) system and the Diagnostic and Statistical Manual (DSM) (Gelder et al, 1983).

2.4. Biochemical Findings in Depression

2.4.1. Cortisol in Depression

Normally, cortisol secretion shows a peak during the day and falls to very low levels during the night; in many severely depressed patients, hypersecretion occurs and the levels remain elevated throughout the 24 hours (Schildkraut, 1978). Since cortisol secretion is regulated by the hypothalamic control on the pituitary gland, a hypothalamic-pituitary-adrenal hyperactivity has been postulated to be a main abnormality in depression (Mendlewicz, Charles and Franckson, 1982).

Administration of dexamethasone suppresses cortisol secretion in normal subjects but fails to do so in some depressive patients (Carroll, Feinberg, Greden, Tarika, Albala, Haskett, McIJames, Kronfol, Lohr, Steiner, de Vigne and Young, 1981; Mendlewicz et al, 1982), when cortisol is determined in plasma within 24 hours. Although it is not totally accepted, the test (dexamethasone suppression test, DST) has been described as a specific state-dependent biological marker of endogenous depression (Mendlewicz et al, 1982). Carroll et al. (1981) set the guidelines for the DST and reported that patients with "melancholia (endogenous depression)" were identified by the test. Using the test, Mendlewicz et al. (1982) showed that primary depressive patients did not suppress cortisol, in contrast to secondary depressive patients and psychiatric controls. In the same work, the test differentiated psychotic from non-psychotic patients but not bipolar from unipolar patients. It was also shown that DST non-suppression might identify individuals at high risk of committing suicide (Robbins and Alessi, 1985).

However, there are controversies about the test that may stem from methodological differences. Carroll et al. (1981) reported no correlation with age or sex but in experiments on rats, a suppressive dose found increased resistance with aging (Oxenkrug, McIntyre, Stanley and Gershon, 1984). Also, whilst a high dose may decrease the sensitivity of the method (Carroll et al, 1981), low doses do not cause suppression (Oxenkrug et al, 1984). Finally, in a study that challenged the efficacy of the method, it was shown that cortisol suppression correlated with dexamethasone levels in serum (Morris, Carr, Gilliland and Hooper, 1986). The failure of the method could be attributed to insufficient dexamethasone levels and consequently intravenous administration might be required in order to screen on an objective basis a population of depressive patients.

To date, no other neuroendocrine system has been shown to be affected as frequently and consistently by depression.

2.4.2. The 5-HT System

The determination of tryptophan in plasma has not led to conclusive evidence. Free and bound tryptophan concentrations were found normal in the plasma of depressive patients (Coppen, 1972), but both free and bound fractions were significantly decreased in endogenous depression compared to neurotic depressive patients and controls (Fiore, Malatino and Petrone, 1979). In a group of psychotic and neurotic patients, total tryptophan was unchanged whereas free tryptophan concentration was higher (Niskanen, Huttunen, Tamminen and Jääskeläinen, 1976). Conversely, a lower concentration of free tryptophan and normal tryptophan levels have been found in

depressed women (Green and Costain, 1979). Thus, reports indicate elevation, decrease or no changes for plasma free tryptophan levels.

The uptake of 5-HT in platelets of depressive patients may be decreased (Modai, Malmgren, Åsberg and Beving, 1986; Green and Costain, 1979) and platelet MAO activity was significantly lower in bipolar depressive patients compared to unipolar depressives and controls (Murphy and Weiss, 1972) or similar between boys with major depressive disorders and normal controls (Rogeness, Mitchell, Custer and Harris, 1985). Apart from being inconclusive, data on peripheral MAO activity would not necessarily indicate that the same results should be expected from brain MAO activity (Schildkraut, 1978). If it did, it could be either an emergency mechanism to conserve 5-HT or equally possibly a reason for reduced 5-HT production (van Praag, 1981).

Post mortem examination of brain tissue from suicides showed that 5-HT levels in hindbrain and raphe were lower or unchanged and 5-HIAA concentration was also found lower or normal (Airaksinen and Airaksinen, 1978; Green and Costain, 1979). Such examinations are, unfortunately, of limited use because time of sampling varies uncontrollably and the method of suicide can confound the results. Also diagnosis is not always obtained and information on medication is not always available, regarding the "patient" before suicide (Abrams, 1978; Hullin, 1976; McIntyre and Stanley, 1984).

Levels of tryptophan and 5-HIAA in the CSF of patients have been determined with or without probenecid pretreatment (section 1.7). CSF sampling from normal volunteers is not undertaken lightly and, consequently, controls are usually patients with neurological diseases or psychiatric patients who have not been diagnosed as

having primary depressive illness. The few studies involving normal controls are particularly highlighted.

Tryptophan concentration in CSF was found unchanged or lower in depression (Airaksinen and Airaksinen, 1978; Green and Costain, 1979; Schildkraut, 1978). Compared to normal controls, tryptophan was found decreased in depressive patients (Coppen, 1972; Mena, Aguado and de Yebenes, 1984). In a rigorously controlled study, tryptophan levels of CSF in manic and depressive patients were not different from controls (Gerner, Fairbanks, Anderson, Young, Scheinin, Linnoila, Hare, Schaywitz and Cohen, 1984).

The status of 5-HIAA is equally equivocal, with CSF 5-HIAA levels being either normal or lowered in depressed patients and, significantly, not returning to control values upon recovery (Abrams, 1978; Airaksinen and Airaksinen, 1978; Green and Costain, 1979). Accumulation of 5-HIAA after probenecid was lower in depressive patients, compared to neurological and other controls (Abrams, 1978; Green and Costain, 1979). However other reports showed no differences between drug-free depressives and inpatient controls, at base line or after probenecid (Abrams, 1978; Bowers, 1972).

At baseline, CSF 5-HIAA levels of endogenous depressive and reactive depressive patients were similar, compared to inpatient controls, but after probenecid, 5-HIAA showed small increases in both psychiatric groups (van Praag, Korf and Schut, 1973). Unipolar depressives showed a greater increase after probenecid than bipolar patients (Abrams, 1978) and also unipolar patients had normal levels of 5-HIAA after probenecid (Bowers, 1972), implying that low 5-HIAA concentration values in CSF, persisting even after probenecid, might

be a characteristic of bipolar patients. Since the evidence is conflicting, however, it is not likely that, at this stage, CSF 5-HIAA levels can be used to discriminate between groups of depressive patients (Ridges, 1976). Higher 5-HIAA levels have also been reported in female depressive patients compared to healthy controls (Koslow, Maas, Bowden, Davis, Hanin, Javaid, 1983). The same study disclosed many differences that are age- or sex-dependent, thus calling for better-controlled studies. The same conclusions about age and sex, though not always in the same direction were reached by Gerner et al. (1984) who also used healthy controls but found no differences in CSF 5-HIAA levels between them and depressive or manic patients. The only change they reported was that GABA levels in depressives were lower than controls (Gerner et al, 1984).

Compared also to healthy controls, euthymic bipolar patients had similar levels of 5-HIAA, and 5-HIAA and HVA levels correlated significantly, indicating that 5-HT and dopamine metabolism might be correlated (Berrettini, Nurnberger, Scheinin, Seppala, Linnoila, Narrow, Simmons-Alling and Gershon, 1985). This correlation has also been shown in the past (van Praag et al, 1973).

Another finding that has been challenged is a bimodal distribution of 5-HIAA in primary depression, distinguishing a group with normal values and one with lower values (Åsberg, Thorén, Träskman, Bertillson and Ringberger, 1976), the latter group being associated with higher likelihood to commit suicide. However, in a study on suicide "attempters" and "non-attempters", 5-HIAA levels were the same in both groups (Secunda, Cross, Koslow, Katz, Kocsis, Maas and Landis, 1986). No relation of CSF 5-HIAA concentration with suicidal history was disclosed in euthymic bipolar depressives

(Berrettini et al, 1985). Interestingly, lower 5-HIAA concentrations in CSF were found in impulsive offenders but not other groups of violent offenders, thus correlating low 5-HIAA with impulsivity rather than violence (Linnoila, Virkkunen, Scheinin, Nuutila, Rimon and Goodwin, 1984b). In a follow-up, patients that exhibited low 5-HIAA levels in CSF during an original study were found to have increased frequency of readmission, compared to patients with normal 5-HIAA, a finding supportive of the existence of a 5-HT-deficient subgroup among depressive patients (van Praag and de Haan, 1979). Unfortunately, this study did not yield information on the subject of suicide prevalence among the two groups.

Finally, 5-HIAA levels did not correlate with clinical state before or after treatment with electroconvulsive therapy or tricyclic antidepressant drugs (Abrams, 1978; Abrams, Essman, Taylor & Fink, 1976). On the other hand, low CSF 5-HIAA and high urinary metanephrine concentrations in unipolar depression were found to correlate with higher response to tricyclic antidepressants (Maas, Koslow, Katz, Bowden, Gibbons, Stokes, Robins and Davis, 1984).

2.4.3. The Catecholamines

The state of catecholamine research is not more conclusive than that of 5-HT.

Tyrosine, the dopamine and adrenaline precursor, had normal plasma levels in depression and dopamine-β-hydroxylase, which converts dopamine to noradrenaline, had similar values between bipolar and unipolar depressives and also between them and normal controls (Lamprecht, Ebert, Turek and Kopin, 1974; Levitt, Dunner,

Mendlewicz, Frewin, Lawlor, Fleiss, Stallone, Fieve, 1976).

In a study of noradrenaline kinetics in the periphery, it was found that plasma NA was elevated in primary depressive disorder and correlated with endogenous depression, while plasma adrenaline was normal (Esler, Turbott, Schwarz, Leonard, Bobik, Skews and Jackman, 1982); the same authors reported an increased peripheral uptake of noradrenaline into neurons and assumed that the mechanism in brain might be similar, leading to lower noradrenaline concentrations at receptor sites.

Post mortem studies have shown no changes in noradrenaline or dopamine levels in brain areas of depressive suicides (Green and Costain, 1979).

Of the noradrenaline metabolites, urinary vanillylmandelic acid (VMA) is thought to be the predominant peripheral metabolite and MHPG the primary brain metabolite (Green and Costain, 1979). It is not clear how much of urinary MHPG is of brain origin, the value being held between 25-60% (Ridges, 1976); nevertheless, it has been shown to be either unaltered or possibly lower in bipolar and schizoaffective patients (Green and Costain, 1979; Joseph, Risby, Crow, Deakin, Johnstone and Lawler, 1985; Schildkraut, 1978).

With respect to CSF, HVA concentration has been shown to have a gradient down the spinal cord, a property not found for MHPG, and its origin can be traced to caudate nucleus; HVA levels showed a trend to decrease in depression (Green and Costain, 1979) though perhaps only in male patients (Koslow et al, 1983). However, other reports found that HVA concentration was unchanged compared to neurological controls (Mena et al, 1984) or healthy subjects (Berrettini et al, 1985; Gerner et al, 1984); lower HVA levels were also found in

endogenous and neurotic depression characterized by motor retardation (Van Praag et al, 1973) or higher in psychotic patients with schizophrenic symptoms (Bowers, 1973).

Finally, MHPG concentration in CSF was found higher in depressive patients compared to healthy controls (Koslow et al, 1983), unchanged (Gerner et al, 1984) and decreased (Green and Costain, 1979; Mena et al, 1984; Schildkraut, 1978).

It would appear almost impossible to draw a definite conclusion as to whether any one of the three systems is affected by or involved with the precipitation or expression of depressive illness, since the data points in all directions. The reasons are many and include, primarily, insufficient standardization of patient classification and multiplicity in laboratory techniques. Thus, for CSF measurements of 5-HIAA, MHPG and HVA concentrations, an increase, decrease or no change has been shown for all three and although trends can be identified, such as the trend for lower 5-HIAA levels in some types of depression, the data is too confusing to support either such isolated concepts or the seemingly ubiquitous monoamine hypothesis of depression. An obvious conclusion that is frequently overlooked is that this variety reflects an equal number of subtypes of depression.

2.5. Therapeutic Approaches to Depression

The lack of insight into the precise causes of depression has inevitably led to the development of therapies that do not cure the disease but rather alleviate the symptoms and prolong the remission phase of the illness (maintenance therapy). Broadly speaking, we can distinguish therapies in three groups. The first group would include

psychotherapeutic modalities (psychotherapy, behaviour and group therapies etc.), which will not be examined here, and also therapies associated with sleep patterns and scheduled exposure to light (sections 4.5 and 4.7). Another approach is electroconvulsive therapy which is the subject of Chapter 5.

By far, the vast majority of depressive patients is treated by a variety of antidepressant drugs. These fall into two broad groups: the monoamine oxidase inhibitors and the tricyclic and atypical antidepressants. The ensuing sections concentrate on the pharmacological actions of the main representatives of the two groups.

2.5.1. Monoamine Oxidase Inhibitors (MAOIs)

The therapeutic effects of MAOIs are considered to be brought about through inhibition of MAO in brain and a consequent increase in the availability of 5-HT, noradrenaline and dopamine for neurotransmission, although which system(s) is responsible for the curative action and which for the side-effects is still debatable (Laakmann, 1983). The pharmacological profile of MAOIs is more complex than that.

Chronic administration of clorgyline initially increases 5-HT concentrations and decreases 5-HIAA levels in brain, but eventually causes a reduction in 5-HT synthesis (Willner, 1985). The firing rate of 5-HT neurons is also reduced, upon continuing exposure to clorgyline (Blier, de Montigny and Azzaro, 1986; Murphy, Garrick and Cohen, 1983). It is thought that the reduction in synthesis and activity are a compensatory process of the neuron in the presence of excessive 5-HT quantities and could involve a feedback mechanism (Murphy et al, 1983; Willner, 1985).

The accumulating amines may be stored in a cytoplasmic pool rather than in vesicles and thus be unavailable for release (Willner, 1985). Still, it has been shown that tranylcypromine and phenelzine increased catecholamine release, an effect which, at least for tranylcypromine, was stereospecific (Youdim and Finberg, 1983). Moreover, it is known that some MAOIs behave as monoamine uptake inhibitors (Laakman, 1983; Willner, 1985) although the effective concentrations for such an action need to be considerably higher than these necessary to inhibit MAO (Youdim and Finberg, 1983).

Chronic administration of some MAOIs reduces the number of $5\text{-}HT_1$, $5\text{-}HT_2$ and also α_2- and β-adrenergic receptors with a time course that resembles the time course required to obtain a therapeutic response (Murphy et al, 1983; Willner, 1985; Youdim and Finberg, 1983). Another process that is claimed to follow the therapeutic time course is the recovery to normal firing rate of 5-HT neurons after initial suppression following chronic clorgyline treatment (Blier et al, 1986).

On a behavioural level, MAOIs attenuate clonidine-induced locomotor response, reverse reserpine-induced sedation, hypothermia and hypotension whereas, on their own, they can cause increases in both locomotion and temperature (Murphy et al, 1983). Also they possess the interesting property of causing total suppression of rapid-eye-movement (REM) sleep in animals as well as man (Murphy et al, 1983).

The use of MAOIs in psychiatry suffers a major drawback because of their side-effect, notably the (in)famous "cheese reaction" and

have lost much ground to the tricyclic antidepressants. However, there are conditions such as the "atypical" depression, the patients with which show a distinct symptom profile (Pare, 1985) and respond very well to MAOIs, provided that their dosage is carefully monitored and regulated (Youdim and Finberg, 1983).

There is still a lot of ground to be covered before the mode of action of MAOIs is fully understood. The delay in onset of therapeutic response, the need for almost total inhibition of MAO before any results are manifested and also the increase of the concentrations of other indirectly acting amines like tyramine and tryptamine, with so far unknown consequences, are but a few of the obstacles towards a clearer picture of the mechanism of action and utility of MAOIs in depression.

2.5.2. Tricyclic and Atypical Antidepressant Drugs

The category of tricyclic antidepressants (TCAs) initially included compounds that had a three-ring structure and were effective in inhibiting the neuronal uptake of 5-HT and noradrenaline (NA). The list has expanded with time to include compounds that have neither a tricyclic structure nor profound uptake inhibitor properties. To this end, it is hardly suprising that the term "tricyclic" (and even the term "antidepressants"!) have been challenged in search of a more appropriate nomenclature (Todrick and Tait, 1976).

The initial concept of monoamine uptake inhibition as a primary mechanism of action for TCAs has been studied in a variety of tissues and conditions, mainly in brain slices and synaptosomes and also blood platelets with in vitro or ex vivo techniques. These studies

not only provided a relative scale of potency in uptake of 5-HT or NA but also helped to specify the selectivity or lack of it in inhibiting one or the other uptake system. Antidepressants with high selectivity for 5-HT uptake inhibition include femoxetine, citalopram, fluvoxamine, indalpine, alaproclate, CGP-6085A and Wy-25093. Selective NA-uptake inhibitors include maprotiline, nomifensine, nisoxetine and desmethylimipramine. Nomifensine is the only compound which has strong dopamine uptake inhibitor properties. Finally, imipramine, clomipramine, amitriptyline and nortriptyline are examples of inhibitors with roughly equal selectivity (Ögren, Ross, Hall and Archer, 1983; Waldmeier, Baumann, Greengrass and Maitre, 1976). A fourth class comprises those drugs which are reported to possess antidepressant activity but low or no uptake-inhibition properties, the main representatives being mianserin, iprindole and viloxazine (Ögren et al, 1983).

The main direct consequence of uptake inhibition is accumulation of monoamines in the synaptic cleft and a resultant reduction in synthesis, utilization and firing rate (Ögren et al, 1983). The potency of TCAs in modifying these mechanisms is roughly related to their potency as uptake inhibitors at each system (Ögren et al, 1983).

Long-term administration, however, tends to increase the NA turnover, possibly via pre- and post-synaptic feedback mechanisms, and mainly through the presynaptic α_2-adrenergic receptor (Ögren et al, 1983; Sugrue, 1981).

In contrast, both acute and chronic treatment with 5-HT-selective uptake inhibitors have the same decremental effects on

presynaptic mechanisms in 5-HT neurons (Willner, 1985), which appears to be a compensatory mechanism, in view of the increased synaptic availability of the neurotransmitter. However, there are no signs of feedback mechanisms operating to restore turnover and firing rate of the neurons following chronic administration (Ögren et al, 1983; Willner, 1985).

Although there is inconsistency in the binding characteristics of TCAs and atypical antidepressants (ADs) on various kinds of binding sites, a few patterns do emerge and may be closely related to the therapeutic action of these drugs. Amitriptyline, imipramine, clomipramine and desipramine all show high affinities for α_1-adrenergic receptors. This property is common also to maprotilene and mianserin whereas iprindole and nomifensine are only weak blockers of ^{3}H-WB4101 (Green and Nutt, 1983; Ögren et al, 1983). Since their affinities for the α_1-receptor are very similar to the concentration range required to inhibit NA uptake, it has been suggested that reduction of α_1 activity may be an equally possible target during treatment.

An opposite picture has emerged for α_2-adrenergic receptors. Mianserin and, to a lesser extent, amitriptyline are the only compounds with high affinity for the ^{3}H-clonidine site (Ögren et al, 1983; Sugrue, 1981). Since mianserin is a weak uptake inhibitor for both NA and 5-HT, its action on a α_2-adrenergic receptors is considered as a chief constituent of its antidepressant action, despite its greater affinity for α_1-receptors (Ögren et al, 1983). Acute but not chronic administration of mianserin both increased MHPG concentration in brain and blocked clonidine-induced decreases of MHPG, a property unique for mianserin among the antidepressant drugs

examined so far (Sugrue, 1981).

Both tricyclic and atypical antidepressants show low affinity for displacing ^{3}H-dihydroxyalprenolol from β-receptors (Green and Nutt, 1983; Ögren et al, 1983). Despite their low affinity, most TCAs reduce the number of β-adrenergic receptors following chronic treatment, a feature in common with MAOIs, ECS and iprindole, but possibly not mianserin (Green and Nutt, 1983; Ögren et al, 1983; Snyder and Peroutka, 1982; Sugrue, 1981). Most TCAs, fluoxetine but not mianserin, and also MAOIs, all decrease the sensitivity of NA sensitive adenylate cyclase, and it has thus been suggested that antidepressant action might be related to adenylate cyclase subsensitivity, developing only after chronic treatment (Sulser, Vetulani and Mobley, 1978). To this end, it is unfortunate that the distribution of adenylate cyclase is extensive and even astrocyte preparations treated with amitriptyline show a decrease in adenylate cyclase function (Green and Nutt, 1983).

The majority of antidepressants show very low affinity for the 5-HT_1 binding site. In contrast, amitriptyline, nortriptyline and mianserin have high affinity for ^{3}H-LSD sites and, along with imipramine and desipramine, also potently displace ^{3}H-spiroperidol from 5-HT_2 sites (Green and Nutt, 1983). As with α_1-receptors, the affinities for 5-HT_2 receptors are in a similar range with concentrations that effectively block 5-HT uptake (Ögren and Fuxe, 1985; Ögren et al, 1983).

Imipramine, clomipramine and desipramine have been reported to reduce the density of ^{3}H-5-HT binding, mianserin and iprindole do not affect it and fluoxetine has yielded equivocal results (Ögren et al,

1983). Overall, it appears that 5-HT_1 binding sites are not among the primary targets of antidepressant drugs.

With respect to 5-HT_2 sites, chronic treatment with desipramine, imipramine, amitriptyline, mianserin and iprindole decreases their number (Blackshear, Martin and Sanders-Bush, 1986; Stockmeier and Kellar, 1986; Snyder and Peroutka, 1982). Mianserin, ritanserin and setoperone down-regulate 5-HT_2 receptors after both acute and chronic treatment (Blackshear et al, 1986; Leysen, Van Gompel, Gommeren, Woestenborghs and Janssen, 1986). The partial agonist activity of mianserin explains to some extent the down-regulation of 5-HT_2 receptors (Blackshear et al, 1986), but in general the explanations should be sought in the compensatory mechanisms that may include presynaptic regulation and influences from other neurotransmitter systems.

Finally, it should be added that many antidepressant drugs show remarkable affinities for histamine-H_1 and muscarinic receptors (Ögren et al, 1983), but do not affect muscarinic receptor density upon chronic administration (Snyder and Peroutka, 1982). It appears that action on muscarinic receptors is not a prerequisite for any antidepressant drug, but when it is present, it is related to their side-effects rather than their therapeutic action (Green and Nutt, 1983; Ögren et al, 1983).

2.6. The Pathogenesis of Depression

The causes of depression are not known. Speculations are based on psychodynamic and biochemical changes, which in turn, dictate the therapeutic approaches. The following section will describe the biogenic amine hypothesis of depression and the difficulties that

have been encountered in applying the theory in the framework of today's knowledge of the biochemistry and pharmacology of the illness.

About thirty years ago, a series of observations were made. Firstly, reserpine, a drug used as a hypotensive could cause a depressive syndrome that resembled some forms of naturally occurring depression (Schildkraut, 1978). Biochemically, it was found that reserpine disrupted the capacity of neurons to store monoamines in vesicles, leading to deamination and decrease in the levels of both 5-HT and catecholamines (Schildkraut, 1978). Also, it was observed that certain drugs showed an antidepressant effect when administered for other conditions. Subsequently, these drugs were found either to inhibit MAO activity or block the neuronal uptake of brain monoamines. These observations formed the basis of the catecholamine hypothesis (Schildkraut, 1965) and indoleamine hypothesis (Lapin and Oxenkrug, 1969) of depression and are today known as the monoamine (or biogenic amine) hypothesis which implicates altered function of 5-HT, NA and recently dopamine in the pathophysiology of depression or, at least, certain types of it (van Praag, 1981).

The biochemical findings that partly support such a view have been briefly reviewed in section 2.4. Since the prevailing notion is one of deficiency in 5-HT, one would expect that 5-HT precursors would have antidepressant potency. Studies on the effects of tryptophan, given alone or in combination with antidepressants, and 5-HTP have only given encouraging results in cases where a 5-HT deficiency was assessed (van Praag, 1981). The data is, however,

equivocal (Hullin, 1976; Shaw, 1976) and it appears that tryptophan is more effective in low doses, since higher doses can induce hepatic pyrrolase which effectively reduces tryptophan uptake by the brain (Green and Costain, 1979). It is worth pointing out that abnormal tryptophan pyrrolase activity in depression has been postulated, suggesting that tryptophan enters the kynurenine metabolic pathway in even higher percentage than normally, at the expense of tryptophan availability to the brain (Hullin, 1976; Ridges, 1976).

Tryptophan depletion in normal subjects significantly lowered mood and impaired proofreading tasks, thus supporting the role of 5-HT in mood regulation and the use of tryptophan in enhancing it (Young, Smith, Pihl and Ervin, 1985). On the other hand, tryptophan administration to two patients on MAOIs caused hypomanic reactions (Goff, 1985).

Reserpine, as seen, can precipitate depressive symptoms. More relevantly, administration of pCPA, which inhibits the hydroxylation of tryptophan to 5-HTP, reversed the therapeutic effect of both tranylcypromine and imipramine (Shopsin, Friedman and Gershon, 1976). Since inhibition of NA synthesis by α-methyl-p-tyrosine (aMPT) did not show a similar effect to that of pCPA in imipramine-treated patients, it appeared that the aetiology of endogenous depression and the therapeutic efficacy of both TCAs and MAOIs were linked to 5-HT function. It is unfortunate that tyrosine administration cannot influence catecholamine synthesis the way tryptophan does, by precursor loading (Eccleston, 1981). L-dopa with or without MAOI pretreatment had no convincing antidepressant effect (Green and Costain, 1979).

2.7. Conclusions

In the conventional approach, the monoamine hypothesis would require that monoamine availability be decreased at the synapse and that, consequently, effective medication increase their availability. The first part is far from proven, even though there are many indications to support it. The second part is disputed by the decreases in monoamine synthesis and function following chronic treatment but can be considered as a secondary effect of increased availability (van Praag, 1981). Three problems, however, still hamper this interpretation: (a) the delayed onset of therapeutic effect; (b) the reduction in sensitivity of the adenylate cyclase system and (c) the effectiveness of drugs that do not possess MAO or uptake inhibitor properties (Stahl and Palazidou, 1986; van Praag 1981).

An alternative theory, which sought to explain the pitfalls of the original hypothesis, postulated that the primary cause in depressive illness could be a hypersensitivity of 5-HT and/or NA receptors, with monoamine deficiency a secondary consequence to that (Bunney, Post, Andersen and Kopanda, 1977; Ridges, 1976; van Praag, 1981). Such a hypothesis would be compatible with the observation that most effective antidepressant therapies down-regulate the number of adrenergic and 5-HT receptors with a time lag that correlates with the appearance of therapeutic effects and that synthesis is suppressed (Bunney et al, 1977; van Praag, 1981).

Arguments against the alternative hypothesis, which might also support the original one, is the effectiveness, albeit limited, of 5-HT precursors to alleviate depression, the absence of antidepressant effect following pCPA or aMPT, and the lack of initial aggravation

when treatment commences (van Praag, 1981).

Such is the current state of theories on the causes and relief of depression. Beyond the simplicity of the monoamine hypothesis, there lie presynaptic events based on feedback regulation, multiple interactions between all the major neurotransmitter systems and post-synaptic mediation of neuronal signals. All these areas would need to be examined very carefully, before any answers contribute significantly to the better understanding of depressive illness.

CHAPTER 3 CIRCADIAN RHYTHMS

3. CIRCADIAN RHYTHMS

3.1. Introduction

The existence of rhythmical changes in natural processes requires no confirmation: simple observations would easily convince the most stringent non-believer. These changes are apparent in natural phenomena like the succession of light and dark, the phases of the moon and the change of seasons. What is not as easily perceptible is an enormous number of rhythmical changes in the organization of the biochemical and behavioural milieu of all living organisms.

These changes are characterized by a period of recurrence which can be anything between milliseconds and years. Depending on their frequency, rhythms are mainly classified as circadian, circalunar and circannual, with frequencies of one cycle per day, 28 days, or 1 year respectively (Aschoff, 1981). This section will focus primarily on circadian rhythms and, to a lesser extent,circannual (seasonal) rhythms in experimental animals and humans, in health and disease. Since a comprehensive review is inappropriate,the interested reader is referred to the illuminating work of Conroy and Mills (1970), Moore-Ede, Sulzman and Fuller (1982) and Palmer (1976).

3.2. Generation and Description of Rhythms

A biological rhythm is defined as the "recurrence of any event within a biological system at more or less regular intervals" (Aschoff, 1981).

The mechanism which controls these rhythms is called the biological clock or pacemaker or oscillator; this is synchronized to external periodic functions which, arbitrarily, are considered

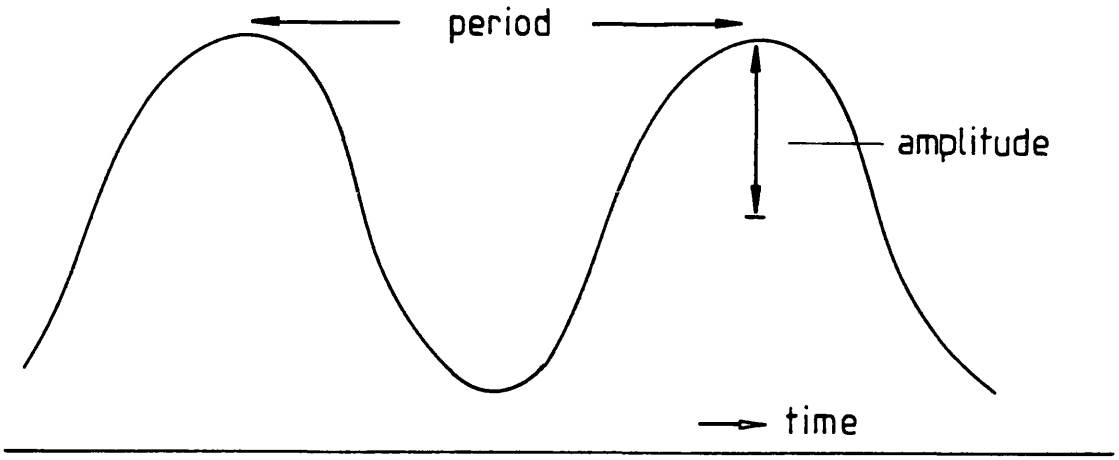
independent. The synchronization will hereafter be referred to as "entrainment" and the external synchronizing stimulus as a "time cue" or "zeitgeber". In the absence of environmental time cues, circadian rhythms present a frequency only approximating 24 h: this emergence of an endogenous frequency is referred to as "freerunning".

Whether the biological clock of an organism is driven by external stimuli or is completely independent and generates its own rhythm is a question that has constructively divided the researchers in this field (Brown, Hastings and Palmer, 1970), but will not be dealt with here.

The oscillation that is responsible for the appearance of circadian rhythms, seems to be generated in a discrete area of the hypothalamus called the suprachiasmatic nuclei (SCN) (Moore, 1983). These nuclei are regarded as the primary, but possibly not the sole, circadian pacemaker and entrain the rhythms of an organism to the environmental succession of light and dark (Moore-Ede, 1983). Since evaluation of light conditions is of paramount importance in synchronization procedures, it follows that a fully functional pathway should include the primary organs of the visual system. Indeed, such a visual pathway, the retinohypothalamic tract (RHT), originates in the retina and terminates in the SCN (Groos, 1982; Moore, 1983). This neural pathway is the primary efferent of information on the sequence of light and dark to the main circadian oscillators, the SCN, for the entrainment of endogenous rhythms that adjust the organism to its environment: the process is ultimately a homeostatic mechanism.

The main characteristics in the description of a rhythm are the following:

Fig. 3.1



(1) acrophase: the lag time from a defined reference point to another defined point in a rhythm, usually the peak as defined by a mathematical model, rather than the time of a possibly fortuitous extreme value.

(2) amplitude: one half of the difference between the highest and lowest point in a rhythm defined by a mathematical model, rather than the difference between extreme values.

(3) frequency: the number of cycles per unit of time.

(4) period: the time interval for one complete cycle (τ and T for the oscillator and the zeitgeber, respectively).

(5) phase: the positional relationship between two or more cycles and also the determinant of any part of the cycle (ψ and ϕ for the oscillator and the zeitgeber, respectively).

(6) phase angle: the phase relationship between any two reference points of two rhythms.

(Halberg, 1980; Moore-Ede, Sulzman and Fuller, 1982; Palmer, 1976).

In order to identify a rhythm, certain methodological criteria must be met. Firstly, in the absence of any identifiable time cues, the rhythms should be able to freerun with a period close to 24 hours. Then, a given factor in the environment should act as a zeitgeber and entrain the rhythm. The rhythm should follow a gradual shift in the phase so that it eventually accomplishes a distinct phase difference. When the zeitgeber is removed, the rhythm should be able to revert to its former phase and period, again in a gradual process. Finally, the intensity and timing of the zeitgeber should affect the period of the rhythm (Conroy and Mills, 1970; Enright, 1981; Moore-Ede et al, 1982).

3.3 Neurophysiology of the Pacemaker

The SCN are anterior hypothalamic nuclei located on either side of the third ventricle and dorsally to the optic chiasm. It was not until recently that the SCN were also identified in humans. Although the evidence is pretty much circumstantial, it seems that the nuclei and their projections are anatomically and histochemically homologous to the SCN of other mammals and indirect pathophysiological evidence suggests that their function is also the same (Moore-Ede et al, 1982; Moore-Ede, 1983). Each SCN is interconnected with the contralateral SCN by distinct projections to and from the equivalent contralateral area. The main projection to the SCN is the RHT which runs along the optic nerve and is always bilateral, usually greater to the contralateral SCN. Other projections to the SCN include the ventral lateral geniculate nuclei and the mid-brain raphe nuclei (Groos, 1982; Moore-Ede et al, 1982; Moore, 1983).

Section of the optic nerve leads to loss of entrainment; section

of visual pathways beyond the optic chiasm leads to blindness but does not affect entrainment. This shows that pathways mediating vision and entrainment function separately (Moore, 1983).

SCN lesions lead to loss of circadian rhythmicity, f.e. of drinking behaviour, activity, adrenal corticosterone levels and temperature (Clarke and Coleman, 1986; Eastman, Mistleberger and Rechtschaffen, 1984; Moore, 1983). This could be interpreted as showing merely that the SCN function as a connective station between retinal input and the real oscillator(s). However, metabolism and multiunit activity in the isolated SCN display a circadian rhythm peaking during the day, a property not found so far in any other examined brain structure (Groos, Mason and Meijer, 1983; Moore, 1983). This is, to date, the most convincing evidence that the SCN are indeed the pacemakers in the mammalian brain. However, as it will be seen later, the SCN may not be the sole pacemaker and areas like the ventromedial nucleus can act as oscillators particularly when the SCN are lesioned or rendered functionally useless.

Of the SCN cells, a large percentage respond to visual stimulation of the retina. The majority of these light-responsive cells change their firing rate tonically when luminance levels are changed. Their discharge is either increased (light-activated) or decreased (light-suppressed). Normally, the threshold for triggering these changes is quite high; moonlight, for example, has intensities that do not affect the SCN (Groos et al, 1983).

There is not much information on the substances that mediate transmission in the SCN. Acetylcholine, vasopressin and 5-HT have been found in significant quantities and may possess a functional

role. Other substances, that have been discovered and may be involved in the circuitry of the SCN include enkephalin, somatostatin, noradrenaline, vasoactive intestinal polypeptide, substance P and avian pancreatic peptide (Jacklet, 1985; Moore, 1983).

Of all these substances, the evidence for 5-HT is best documented. Its content in the SCN is high in terminals which have their cell bodies in the mid-brain raphe complex. Both 5-HT and 5-HIAA concentrations show a circadian variation in this area and tryptophan hydroxylase is also present. On the other hand, raphe lesioning or destruction of 5-HT neurons by 5,7-dihydroxytryptamine (DHT) in the SCN does not abolish rhythmicity, implying that 5-HT may be a neurotransmitter in the SCN mediating the expression rather than generation of circadian rhythms (Groos et al, 1983).

There is an extensive output network that connects the SCN with other regions of the hypothalamus and also other areas of the CNS, frequently in a bilateral modality (Fig. 3.2). The main pathways are to the paraventricular nucleus, the retrochiasmatic region, the lateral hypothalamus, the ventromedial nucleus and other regions of the tuberal hypothalamus, the midbrain and also the brainstem and part of the spinal cord (Groos, 1982; Moore, 1979; Moore, 1983; Moore-Ede et al, 1982).

It is of particular interest to examine the pathway that includes the spinal cord since it seems to be involved in the feed-back regulation of the SCN. Thus, a projection innervates firstly the intermediolateral cell column of the spinal cord and, from there, the preganglionic section of the superior cervical ganglion (Groos, 1982). The latter has two distinct effects.

The first is postganglionic, possibly noradrenergic, innervation

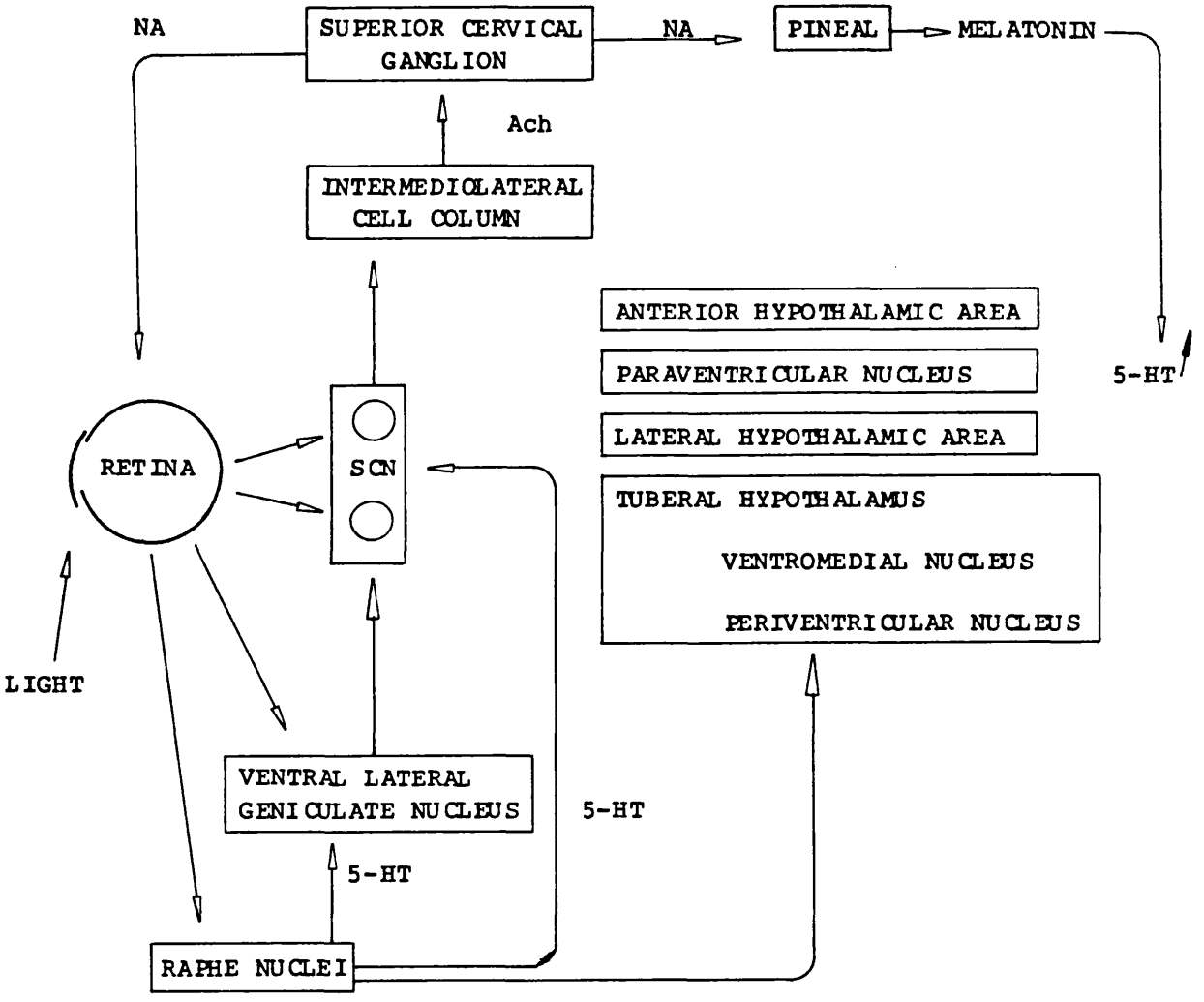


Fig. 3.2 DIAGRAMMATIC REPRESENTATION OF KNOWN SCN CONNECTIONS. 5-HT: 5-hydroxytryptamine; NA: noradrenaline; Ach: acetylcholine.

of the retina. The effect may be both direct, by imposing a circadian rhythm in the sensitivity to light, and indirect, by dilating the pupil, thereby allowing more photic energy to reach the retinal photoreceptors (Groos, 1982).

Secondly, it innervates, again via sympathetic postganglionic output, the pineal gland. Stimulation of β-adrenergic receptors in the pineal induces a rhythmical activity of N-acetyl-transferase (NAT) which, in turn, regulates the rhythmical production of melatonin (Jacklet, 1985).

Elevated melatonin concentration causes an increase of 5-HT levels in the diencephalon whereas pinealectomy has the opposite effect. Thus, there is a possibility that the SCN regulate their input from the raphe nuclei and the lateral geniculate nuclei, both of which contain 5-HT innervations to the SCN, by controlling melatonin rhythm and production. However, neither sympathectomy nor pinealectomy affect the normal entrainment or the expected phase responses to external stimuli in freerunning animals. Although these effects are negative, the presence of 5-HT and melatonin in both the SCN and the pineal, and also their interaction, is strongly suggestive of an operational link in the regulation of photoperiodic phenomena (Groos, 1982).

3.4. The Pineal Gland and Melatonin

Since the pineal has been mentioned by way of examining SCN outputs, it is worth considering its involvement in the generation of circadian rhythms.

The pineal and its primary product, melatonin, are certainly

involved in avian regulation of rhythms (Menaker and Binkley, 1981), although the mode of action has yet to be identified.

In rodents, melatonin and its precursor, NAS, rise abruptly during the dark phase, as measured in serum, pineal and whole brain (McNulty, Prechel and Simmons, 1986). The levels of melatonin are selectively higher in the hypothalamus and lower in midbrain and cerebellum (Pang and Brown, 1983). Chronic administration of the MAO inhibitor, pargyline, increased its levels in rat pineal (Wirz-Justice, Kafka, Naber, Campbell, Marangos, Tamarkin and Wehr 1982b) and a single oral dose of tranylcypromine had a similar effect in humans (Oxenkrug, McIntyre, Balon, Jain, Appel and McCauley, 1986). It is not known whether these MAO inhibitors exerted their effect by blocking the light suppression, by direct stimulation of the β_1-adrenergic receptors of the pineal or by increasing the levels of noradrenaline (NA) and 5-HT. Pineal 5-HT and 5-HIAA and also noradrenaline and dopamine exhibit a circadian rhythm with higher levels during the light and lower levels during the dark phase, in complete contrast to melatonin (Tang, Hadjiconstantinou and Pang, 1985).

The pineal does not seem to regulate circadian rhythmicity and pinealectomy does not abolish rhythms in mammalian brain. Following SCN lesion, melatonin did not restore the abolished rhythm of drinking in the rat (Cassone, Chesworth and Armstrong, 1986). In contrast, sham-lesioned animals or animals with incomplete lesions, following 6-OH-dopamine or 5,7-DHT did entrain their rhythm to the period of the injection regime, only when the onset of the free-running rhythm coincided with the entraining stimulus (Cassone et al, 1986). The obvious assumption is that melatonin function requires

that the SCN be functionally intact.

Melatonin can be considered as a hormone with positive but, for the time being, obscure connections with the circadian system,and a promising field for research.

The pathways that have so far been identified in the circuit revolving around the SCN are presented in a concise form in Fig. 3.2.

3.5. Freerunning Rhythms and Their Controlling Mechanisms

3.5.1. Animal Experiments

Circadian rhythms have been described for physiological processes as overt as body temperature, sleep and heart rate, and as discrete as enzyme activity and hormone secretion. Rhythms have also been found in drinking, feeding and many other aspects of behaviour.

The generation of circadian rhythms has so far been attributed to the functions of the SCN. To this end, bilateral lesion of the SCN in rat brain inhibited the appearance of freerunning rhythms in temperature and sleep-wake that were expected when the animals were left in constant dim light. Partial lesioning allowed the generation of weak freerunning rhythms (Eastman et al, 1984). This kind of data would support the idea that one pacemaker drives both rhythms.

However, there are a lot of data which amply challenge that conventional view and indicate instead that either the basic pacemaker has different coupling mechanisms and capacities for each rhythm or that more than one oscillator, not necessarily within the SCN, share the task of regulating the rhythmicity of living organisms.

Such a conclusion was arrived at in an experiment in which rats

were maintained in constant light, the intensity of which was increased in steps. This led to the disappearance of the sleep-wake rhythm, followed after about 2 weeks by the disapperance of the temperature rhythm (Eastman and Rechtschaffen, 1983). Continuous exposure to constant light is known to produce a separation or even disintegration of freerunning rhythms (Eastman et al, 1984; Mitler, Lund, Sokolove, Pittendrigh and Dement, 1977). The difference in timing of the uncoupling of the two rhythms suggested that either two separate oscillators or a common oscillator with either two subunits or different coupling strengths regulate the two rhythms (Eastman and Rechtschaffen, 1983; Mittler et al, 1977).

The same problem has been examined by altering not only the visual information of the animals but also other parameters that can affect their behaviour.

In an experiment in which drinking behaviour and wheel running activity in the rat were examined under conditions of food deprivation alternating with ad libitum feeding, the data showed that meal-associated drinking behaviour was controlled by an oscillator other than the SCN, since lesion of the latter did not abolish that rhythm. Wheel-running activity, which is consistently associated with the SCN, was also influenced by the rhythmicity of the meal-associated oscillator (Clarke and Coleman, 1986).

Restrictions in water availability to rats maintained in normal light-dark cycles did not show the potency of food availability as a zeitgeber and the animals did not re-entrain to a rhythm dictated by it (Mistlberger and Rechtschaffen, 1985).

Food restriction provokes an anticipatory wheel-running activity with a rhythm that does not depend on the SCN: lesion of the latter

did not affect this rhythm, which could also be observed concurrently with light-entrained or freerunning activity rhythms (Rosenwasser, Pelchat and Adler, 1984; Stephan, 1986b). In this context, rats on food restriction were allowed free access to food while simultaneously undergoing a change of light conditions (phase shifting or freerunning). When food deprivation was reintroduced, the wheel-running activity associated with it reappeared in the same temporal relation that that activity had, when compared to the original rest-activity rhythm (Rosenwasser et al, 1984). This data suggested that the food-anticipatory activity rhythm is co-ordinated by an oscillator other than the SCN, capable of coupling to it and also capable of showing "memory", i.e. expressing itself anew, in phase with the main oscillator and in conditions that somehow relate to the original conditions during its appearance.

In a series of experiments based on the same principle, rats undergoing food restriction and changes in the light-dark cycle showed that the rest-activity cycle obeyed the light signals whereas anticipatory activity entrained to food presentation. The two rhythms did not entrain to each other if the difference in their period exceeded ten minutes (Stephan, 1986a). This finding was surprising in view of the fact that both rhythms have demonstrated their intrinsic ability to entrain over a much wider difference of periodic presentation of a stimulus. Nevertheless, similar data lent support to the notion that the two rhythms are driven by different oscillators, mutually but loosely coupled, with the light-entrained oscillator being slightly dominant (Stephan, 1986b).

3.5.2. Studies in Humans

Humans, understandably, are not as appropriate for experiments that require this kind of manipulation and self-sacrifice. Still, a considerable number of studies have been conducted with volunteers who remained in social isolation for considerable lengths of time in caves or purpose-built bunkers, even though, usually, they had full control of arranging their light-dark cycle.

When these volunteers were left isolated, their rhythms began to freerun initially with a common period, longer than 24 hours. Progressively their rhythms split into two distinct groups. Each group freerun with a period different from the initial (Conroy and Mills, 1970; Moore-Ede, 1983; Palmer, 1976; Wever, 1979).

These two groups typically contained the following rhythms. The first group included REM sleep propensity, plasma cortisol, urinary potassium excretion and followed the rhythm of core body temperature; the second group comprised of slow-wave sleep, skin temperature, plasma growth hormone, urinary calcium excretion and obeyed the period of rest-activity rhythm (Moore-Ede et al, 1982). The first group of rhythms presumably is driven by a pacemaker whose coupling strength was calculated to be about four times stronger than the pacemaker that regulates the second group. This last pacemaker is thought to be the SCN (Moore-Ede, 1983).

The data presented here from experimental animals and human subjects clearly justifies the belief that the SCN are not a single pacemaker unit in the organism. Still, a lot more research is required before it can be claimed with certainty whether there is one multi-unit pacemaker, two or more pacemakers with equal or unequal coupling strengths upon each other or two or more pacemaker with

varying coupling strength upon minor oscillators or rhythms.

3.6. PHARMACOLOGICAL INTERVENTION IN CIRCADIAN RHYTHMICITY

For a long time, it was believed that circadian rhythms were not susceptible to changes due to drug administration and modifications of the rhythm parameters were brought about by alterations in the illumination and food and water availability. The only substances which had been shown to modify circadian rhythms were some hormones (oestrogen, progesterone, testosterone and melatonin), carbachol, a-bungarotoxin and, notably, deuterium oxide (Hastings, 1970; Wirz-Justice and Campbell, 1982).

Lately, however, a few more substances were found to be capable of changing rhythm parameters. The following section will deal with the effects of these drugs on rhythms.

3.6.1. Drugs and Locomotor Activity

It is established beyond doubt that nocturnal rodents display a circadian pattern in spontaneous locomotor activity which is much more pronounced during their active phase, the dark part of the light-dark cycle (Rosenwasser et al, 1984; Summer, Ferraro and McCormack, 1984; Mistlberger and Rechtschaffen, 1985; Stephan, 1986a; 1986b).

Typically, this rhythm shows two components: one occurring at the end of the light phase and dominating the dark phase and a second component that reaches a peak towards the end of the dark phase or the beginning of the light phase (Hutchins and Rogers, 1973).

Deuterium oxide was long known for lengthening the period of a

freerunning activity rhythm (Palmer, 1976). Chronic administration of clorgyline also lengthened the period of the activity rhythm of hamsters in constant dark and also delayed the onset of nocturnal running under normal light-dark conditions (Wirz-Justice, Groos and Wehr, 1982a). The same effect was seen when clorgyline was administered directly to the SCN (Wirz-Justice et al, 1982a). Imipramine promoted dissociation of the activity rhythm and possibly also slowed the rhythm in hamsters. In common with clorgyline, it too, abolished the freerunning rhythm of food intake in blinded rats, when administered in or very near the SCN. Lithium carbonate shared the same properties with imipramine and clorgyline (Wirz-Justice et al, 1982a). Thus, a tricyclic antidepressant, a MAO inhibitor and an antimanic-antidepressant drug have in common the property to lengthen the freerunning activity rhythm. The other thing they have in common is their effects on 5-HT system. Considering that 5-HT is found abundantly in the SCN, Wirz-Justice et al (1982a) were prepared to suggest that the effect of period lengthening of these drugs could be connected with action on 5-HT function in the brain and particularly the SCN.

In contrast, imipramine, along with clomipramine and zimelidine caused a breakdown in the freerunning activity rhythm of the rat (Redfern and Martin, unpublished observations). Thus, whilst Wirz-Justice et al (1982a) considered that the therapeutic effect of antidepressant drugs might rest with their ability to cause a phase delay as a counterbalance to the phase advance most often encountered in depression, Redfern and Martin postulated that the complete breakdown of the rhythm facilitated the de novo resynchronization at a corrected phase angle.

It is noteworthy that spontaneous activity may become arrhythmic or split into two components after long exposure to bright light or constant dark and other conditions (Eastman and Rechtschaffen, 1983; Eastman et al, 1984). Moreover, the two components may be driven by different oscillators which also employ different neural pathways. Depletion of cerebral catecholamines by a-methyl-p-tyrosine and of 5-HT by pCPA selectively abolished the two phases of locomotor activity in mice (Hutchins and Rogers, 1973). Additional support is lent to this suggestion by the finding that prolonged illumination caused a desynchronization in the locomotor activity and also REM and non REM sleep rhythms: episodes of activity were interpersed in the inactive phase and REM and NREM episodes in the active one (Mitler et al, 1977).

The data suggest that the locomotor activity rhythm which was once thought to be exemplary in its clarity and consistency should be approached with caution with respect to its origin and significance.

3.6.2. Drugs and Receptor Rhythms

In a series of binding studies, it was shown that α- and β-adrenergic, muscarinic, opiate and benzodiazepine receptors in rat forebrain and dopamine and benzodiazepine receptors in rat striatum displayed a circadian rhythm in their number, B_{max}, but not their affinity, K_D (Kafka, Wirz-Justice and Naber, 1981; Naber, Wirz-Justice and Kafka, 1982; Wirz-Justice et al, 1982b; Nowak, Mogilnicka and Klimet, 1986).

These receptor rhythms were found to be vulnerable to the same agents that modified activity rhythms. Thus, chronic treatment with clorgyline generally caused a phase delay in the rhythm of these

receptors (Wirz-Justice et al, 1982b). Interestingly, the effect on receptor number was time-dependent: the drug increased receptor population at one time of the day and decreased it at another. Imipramine, like clorgyline, delayed the phase position of the same receptor populations. Lithium, on the other hand, phase-delayed the rhythms of cholinergic, opiate and striatal benzodiazepine receptors but abolished the rhythms of forebrain α- and β-adrenergic and benzodiazepine receptors (Kafka, Wirz-Justice, Naber, Moore and Benedito, 1983). Finally, fluphenazine, a drug used in schizophrenia and related psychoses also phase-delayed receptor rhythms (Naber et al, 1982).

The effects of these drugs on the 24 hour mean value of receptor ligand binding were more variable. Clorgyline reduced binding over 24 h in striatal dopamine and benzodiazepine receptors and forebrain muscarinic receptors (Wirz-Justice et al, 1982b). Imipramine reduced the 24 h mean of most receptor numbers and lithium increased the number of all receptors except for forebrain benzodiazepine and striatal dopamine receptors, the numbers of which were reduced (Kafka et al, 1983). The effects of fluphenazine resembled those of lithium; it increased the number of α- and β-adrenergic, muscarinic and opiate receptors and markedly reduced dopamine receptor numbers (Naber et al, 1982).

A finding that must be added to this list is the existence of seasonal rhythms in binding to α- and β-adrenergic, muscarinic, opiate and dopamine receptors (Kafka et al, 1983).

The demonstration of circadian and seasonal rhythms in receptor numbers indicates that the response to a drug may be modified

depending on the time of the day it is administered and that, possibly, administration time might need to be corrected for seasonal changes. These rhythms should not be treated as an independent finding; that is, the origin of this variation should be identified and correlated to parallel changes in the metabolic pathways of the transmitters that bind to these receptors.

In conclusion, three drugs used in psychiatric disorders share the ability to phase-delay rhythms in locomotor activity and receptor numbers. No explanation can be given for the changes in the 24 h means.

3.6.3. Circadian Rhythms and 5-HT Function

The organization of the serotonergic system has been studied quite extensively and it can be said with certainty that many of its components display a circadian rhythm.

In the periphery, free tryptophan concentrations in rat plasma showed a rhythm with peak and trough at the beginning of the light and dark phases, respectively (Redfern and Martin, 1985). Chronic administration of imipramine lowered free tryptophan levels and abolished the rhythm, whereas clomipramine and zimelidine increased the concentration and caused a phase-delay (Redfern and Martin, 1985). The tryptophan rhythm in plasma was abolished when feeding was stopped altogether in rats on restricted feeding schedule (Ho, Chic and Brown, 1985), but was only phase-shifted, compared to controls, in another study when restricted feeding was imposed (Morgan and Yndo, 1973).

Both tryptophan and 5-HT concentrations in rat brain vary significantly over 24 hours (Brown, Nicholass and Redfern, 1979;

Fernstrom, 1978; Morgan and Yndo, 1973). The rhythms for brain tryptophan and 5-HT do not necessarily follow the rhythm of tryptophan in plasma, denoting that possibly the variation in brain is independent from the variation in plasma (Morgan and Yndo, 1973), despite the fact that the actual brain levels are in many ways related to plasma tryptophan concentration (Chapter 1).

The findings that pCPA abolished the brain 5-HT rhythm and that the hyperactivity response to the administration of tryptophan and pargyline, but not to 5-MeODMT, was characterized by a circadian variation, suggested that the rhythm for 5-HT concentration depended on its synthesis (Brown et al, 1979). The hypothesis was not substantiated since it was found that tryptophan hydroxylase activity in whole rat brain did not possess a circadian rhythm (Brown et al, 1979; 1982). Significantly, however, Cahill and Ehret (1981) reported that tryptophan hydroxylase activity in brain stem is characterized by a diurnal variation, peaking at the end of the dark phase.

Binding of ^{3}H-5-HT in rat brain was characterized by a rhythm peaking in the late light and mid-dark phases (Weseman, Weiner, Rotsch and Schultz, 1983) whereas binding of ^{3}H-imipramine was increased at the end of the dark and decreased at the end of the light phase (Briley, 1985). The diurnal variation in receptor binding is reflected by the variation in responses mediated by those receptors. Thus, the 5-HT_2 receptor-mediated head-twitch response in the mouse exhibited such a diurnal variation whereas the 5-HT syndrome, the expression of which is mediated mainly by 5-HT_1 receptors, did not.

Finally, 5-HT concentration were reported to display a

significant variation over 24 hours in monkey CSF (Taylor, Garrick, Burns, Tamarkin, Murphy and Markey, 1982). In contrast, no such variation was reported in rat CSF for tryptophan or 5-HIAA, although 5-HT turnover did exhibit a diurnal rhythm (Hutson, Sarna and Curzon, 1984).

3.7. Human Circadian Rhythms

It is considered appropriate at this point to return to the examination of the human circadian system before arriving at the final conclusion.

The list of functions of a human organism that have been shown to display a circadian variation is lengthy and includes: temperature; cortisol; growth hormone; sex hormones; adrenaline; insulin; urinary excretion of electrolytes; metabolic rhythms; heart rate; pain tolerance; sensitivity to drugs; and blood pressure (Conroy and Mills, 1970; Palmer, 1976).

The last rhythm to be examined is human performance. Testing performance in search for rhythms is hindered by two main obstacles: first the designing of tests that can selectively assess different aspects of alertness and skill and, secondly, the obvious handicap when testing requires that the subjects be woken up. Despite all these, rhythms for reaction time and mental and motor skills have been described (Conroy and Mills, 1970; Colquhoun, 1981), with mental skills peaking around midday and motor skills in the afternoon. It is extremely difficult to attribute the origin of such rhythm to any particular aspect of the human circadian rhythmicity. To this end, association of rhythm in time perception and psychomotor performance with the in-phase rhythm of body temperature (Palmer 1976) can be

described as coincidental. When a subject was entrained to a 25.8 h day, different tasks followed either the sleep-wake or the temperature cycle when these two, predictably, split. This kind of evidence has led to the conclusion that human performance does have a circadian rhythm but different tasks might be controlled by different oscillators and be independent of other overt processes (Monk, Weitzman, Fookson, Moline, Kronauer and Gander, 1983; Folkard, Wever and Wildgruber, 1983).

One important reason for considering the importance of rhythms in performance stems from the problems that two sections of the human population face. First the number of shift workers has increased significantly in the recent decades, with the growing demand to man hospitals, industries, radar controls, mass communications and media stations, power centres and military bases round the clock. Another class of people includes those who are obliged to travel across time zones, like the personnel of airline companies, diplomats and businessmen.

Although the degree of the problem varies among these two classes, subjects in both classes have to be phase-shifted. In this case, they undergo phase advances or delays in both their bodily functions and their performance. Thus, imposing abnormal work schedules or travelling non-stop along time zones can have deleterious effects that not only lead to errors but can jeopardize human lives (Conroy and Mills, 1970; Moore-Ede et al, 1982).

3.8. Conclusions

The mammalian organisms display a circadian rhythm in most of their functions. These rhythms are generated within the CNS and are responsible for keeping the organisms in harmony with their environment. In more practical terms, the changes in receptor, neurotransmitter and metabolic function with time should be taken into account in the diagnosis of illnesses, and the susceptibility of the body to the effects of pharmacological treatment.

This chapter was concerned with the physiology of the circadian system and its manipulation in, presumably, healthy organisms. In the next chapter, we shall examine the alterations in circadian and circannual rhythms that appear to be related to the pathology of affective disorders.

CHAPTER 4 CIRCADIAN RHYTHMS IN DEPRESSION

4. CIRCADIAN RHYTHMS IN DEPRESSION

It was mentioned above (section 2.3.1) that among the prevailing symptoms of depressive illness are a tendency for patients to wake up abnormally early in the morning and to display a mood swing during the course of the day. These, along with the observation that manic depressive illness manifests itself in a rhythmic manner, prompted research into the possibility that abnormalities in the organisation of circadian rhythms are involved in the pathophysiology of affective illness.

It is, thus, considered justifiable to examine the nature of these symptoms and also cite various other abnormalities in circadian organisation that have been reported in depression.

4.1 Diurnal Mood Variation

Depressed patients frequently complain of bad mood during the morning and report a significant alleviation during the course of the day and especially the evening (Wehr, Sack, Rosenthal, Duncan and Gillin, 1983). However, the inverse pattern with gradual worsening towards the evening has also been observed (Kripke, 1983).

4.2. Sleep Abnormalities

Sleep abnormalities are much better documented than any other sign of circadian disorganisation in depressive patients. Since sleep is a very complex process, a brief outline of its main characteristics might be of benefit as an introduction.

The average adult sleeps about 8 hours each night. Sleep consists of two qualitatively different kinds: the rapid-eye-movement sleep (REM or paradoxical sleep) and the non-REM sleep (NREM or

orthodox sleep). NREM sleep is further divided into 4 stages, characterized by increase in depth of sleep and decrease in autonomic activities. Stages 1 and 2 usually precede REM sleep and stages 3 and 4 are the deepest stages of sleep, collectively known as slow wave or delta sleep (Chen, 1979).

REM sleep is characterized by a 90-minute cycle: the time between falling asleep and its onset, and the interval between the first and second episode of REM sleep are both about 90 minutes. REM sleep accounts for about 20-25 per cent of total sleep and there are three to six episodes in each sleep cycle. REM sleep tends to dominate the second half of the cycle (Chen, 1979). Sleep variables are influenced by age, sex, exercise, fatigue, diet and mood and thus may be dependent on and/or interactive with psychobiological aspects of an individual (Chen, 1979). Moreoever, REM sleep appears to be governed by an oscillator since it has been shown to display a circadian rhythm in conditions of temporal isolation or in subjects sleeping during normal daytime (Wehr and Goodwin, 1983b).

According to these generally accepted characteristics of sleep, depressive patients show marked differences in their sleep organization compared to controls, the most persistent of which are difficulty in falling asleep, short REM latency, reduced REM sleep, reduction of stage 4 sleep, increased time spent awake and early morning wakening. None of the above is necessarily found in a depressed patient; some of them, like reduced REM sleep, may be found in theoretically normal groups of people like the elderly and are of questionable value as diagnostic variables (Chen, 1979).

In depressive patients REM sleep episodes tend to occur more

frequently in the first half of sleep, the first of them tends to be longer and have a shorter latency (Wehr et al, 1983).

Unipolar and bipolar depressive patients do not present the same picture with regard to sleep, but short REM sleep latency is fairly common in both conditions, and differentiates these two groups from other groups with affective disorders (Kupfer, 1976).

More detailed analysis of sleep variables, finally, has been shown to aid the recognition of various sub-types of affective illness with considerable accuracy and obvious consequent benefits in treatment (Chen, 1979).

Thus, REM sleep is a variable characterized by a circadian rhythm which appears to be phase-advanced in depression, the most consistent finding being short REM sleep latency. Yet, the fact that the latter, accompanied by smaller than normal differences between daytime and nightime values of body temperature (decreased amplitude), has been reported for depressive patients, as well as remitted patients and normal subjects, would question the usefulness of the finding as a marker for depression (Schulz and Lund, 1983).

The second consistent finding in depressive patients is early morning wakening. This symptom is more common among unipolar than bipolar depressive patients and indicates that the rhythms, which may control waking, are phase advanced compared to a normal light-dark cycle (Wehr and Goodwin, 1983a; Wehr et al, 1983).

4.3. Temperature

The temperature rhythm shows a peak towards the end of the day and a trough during the second half of sleep in normal subjects. In depressive patients the trough is found in the first half in 50% of

the cases (Wehr and Goodwin, 1983b). This does not imply that the patients fall into two distinct groups since patients showed a peak on either half of the sleep period during that study (Wehr and Goodwin, 1983b).

The case of phase-shift in temperature rhythm is, indeed, debatable. Evidence has been accumulating that indicates a trend for the rhythm to phase-advance (Wehr and Goodwin, 1983b). On the other hand, other reports failed to find any differences compared to control groups apart from a possible increase of the mean values (Pflug, Johnsson and Martin, 1983) or amplitude (Lund, Kammerloher and Dirlich, 1983). Remarkably, the temperature rhythm appeared to be normal during the manic phase but totally desynchronized during the depressive phase of bipolar patients (Nikitopoulou and Crammer, 1976).

Considering that body temperature and REM sleep are thought to be driven by the same oscillator (Moore-Ede, 1983), it is rather unexpected to observe such differences in the documentation of phase-advance for the two rhythms, suggesting that the two rhythms may not be causally related (Schulz and Lund, 1983). Still, the two rhythms shifted in phase in normal volunteers under temporal isolation (Weitzman, 1983) and also in a case study in which the rhythms of a manic depressive woman shifted in parallel during both the manic and the depressive phase (Wehr and Goodwin, 1983b).

4.4. Cyclicity

Affective disorders are intrinsically characterized by rhythmicity. Unipolar depression does show cycles in recurrence but the feature is more prominent in bipolar depression where the cycle

can last days, weeks, months or years. So, there is an inherent tendency for the illness to recur.

Moreover, there is the interesting phenomenon of 48 hour cycles in manic depression. Patients typically switch from depression to mania when they experience one of these cycles, which is characterized by a sleepless night following a night of normal sleep (Wehr and Wirz-Justice, 1982; Wehr et al, 1983; Welsh, Nino-Murcia, Gander, Keenan and Dement, 1986), although this cycle is not a prerequisite for the switch to mania. The latter can also be induced when patients are deliberately deprived of one night's sleep.

Similar 48-hour cycles have been shown to regulate the mood of unipolar depressive patients (von Zerrsen, Dirlich and Fischler, 1983) and can also be produced in normal subjects under conditions of temporal isolation (Kripke, 1983), which may suggest that the appearance of 48-hour cycles is due to internal desynchronization of the main oscillators driving the temperature and sleep-wake rhythms, respectively.

4.5. Seasonality

In a recent review of the available literature, it would appear that there is an increased incidence of depression in spring and autumn or during summer time; bipolar illness also showed a seasonal variation, but not as consistent (Rosenthal, Sack and Wehr, 1983). Since the data suggests that light is capable of entraining biological rhythms in humans as it does in other animals (Lewy, 1983), it could be expected that humans, too, may use light to "recognize" the seasonal variation in day length (photoperiodism), mainly through the pineal gland. At first glance it would seem

incompatible that depression is more common during the months of increased sunlight. A possible argument is that higher incidence of depression is reflected by hospital admissions which would not necessarily coincide temporally with the onset of the illness.

On the other hand, a proposed model of supersensivity to light in depression could clarify this point (Lewy, 1983; Lewy, Nurnberger, Wehr, Pack, Becker, Powell and Newsome, 1985). This model was supported by the finding that exposure to bright light during the sleep period of bipolar euthymic patients, when awoken, suppressed plasma melatonin twice as much as control subjects. If there is such a supersensitivity, daylight, which normally adjusts the intrinsic period to 24 hours, might act as a more potent zeitgeber resulting in a phase-advance of the rhythms, a state commonly describing circadian rhythms in depression. The same hypothesis would partly explain the findings that exposure to bright light at certain times during the 24 hours has antidepressant effects (Kripke, 1981; Rosenthal et al, 1983). Such results should be interpreted with caution since Kripke (1981) found that red light also had antidepressant effect under the same experimental conditions, an indication that the crucial factor might be the phase of the interrupted sleep-wake cycle or certain susceptibility of the organization at the particular time.

The importance of light could be reduced when it is considered that blind subjects may have longer than 24 hour periods but also normal 24 hour rhythms in phase or phase-shifted, relative to the normal day (Moore-Ede, Czeisler and Richardson, 1983). This indicated that other cues, mainly socioenvironmental can also

synchronize the oscillators to the external environment.

4.6. Circadian Rhythms of Neurotransmitter and Hormonal Function in Depression

One of the major metabolites of noradrenaline, MHPG, has been measured in urine of normal subjects and depressed bipolar patients (Wehr and Goodwin, 1983b). The levels of MHPG exhibited a circadian rhythm in both patients and controls, and the peak of the rhythm was advanced by several hours in depression (Wehr and Goodwin, 1983b; Wehr and Wirz-Justice, 1982). A phase advance has also been reported for urinary HVA, vanillic acid and 5-HIAA whereas phase-delay was found for vanilyl mandelic acid in depressive patients (Wehr and Goodwin, 1983b).

Total tryptophan also exhibited a diurnal variation in controls and bipolar patients in both neutral and depressive state. There was no difference between controls and patients in either state (Dam, Mellerup and Rafaelsen, 1984). In another 24 hour study total tryptophan was also found unchanged between controls and severely depressed patients but free tryptophan was significantly elevated (Niskanen et al, 1976), although from their data it is probable that neither substance fluctuated significantly during the 24 hours.

Interestingly, in a study that was thoroughly analyzed for circadian variations, both free and total tryptophan exhibited a rhythm in controls and depressive patients (Malatino, Fiore, Costa, Calandra, Petrone and Cacciola, 1982), with elevated levels during the night for the controls, whereas in the study of Dam et al (1984) the peak was found during the light phase. Still, Malatino et al (1982) showed that the peak for the patients was shifted almost 12 h and

considered this as an explanation for the mood swing.

It has been claimed that platelet 5-HT uptake is reduced in depressed patients and that it exhibits a rhythm in both controls and patients (Egrise, Rubinstein, Schoutens, Cantraine and Mendlewicz, 1986; Modai et al, 1986). Uptake of 5-HT also seems to display a seasonal variation in normal subjects and depressive patients; in one study no significant or consistent difference was reported between patients and controls, with both groups reaching a peak in Vmax during winter and spring (Egrise et al, 1986), whereas in another study Vmax was found to be lower in depressive patients throughout the year but reached higher values in both groups during autumn and winter (Arora, Kregel and Meltzer, 1984).

Finally, data for urinary 17-ketosteroids, plasma cortisol, melatonin and serum prolactin are also inconclusive with both phase-delays and advances reported by different workers (Mendlewicz et al, 1982; Wehr and Goodwin, 1983b).

4.7. Antidepressant Therapies and Circadian Rhythms

The results quoted so far could lead to the following three conclusions. First, depression is characterized by abnormalities in circadian organization. Secondly, the abnormalities are usually expressed as phase-advances from the normal rhythm; and thirdly, alterations in sleep pattern are central to the pathophysiology of the illness.

One way to test the strength of such a scheme was to apply treatments that would, in theory, compensate or readjust the putative malfunctions. In this context, sleep therapies have been used for

the alleviation of depression. These therapies range from total sleep deprivation, REM sleep deprivation, partial sleep deprivation of the first half, partial sleep deprivation of the second half to phase advance of the sleep period (Gillin, 1983).

Sleep deprivation is over 50% effective in endogenous depression but the beneficial effects are short-lasting (Gillin, 1983). The treatment is limited by the observation referred to above, that it can promote the switch from depression to mania in bipolar patients.

Partial sleep deprivation is more effective and better tolerated than total sleep deprivation (Gillin, 1983). Also, the existing evidence indicates that deprivation of the first half of sleep is not as effective as deprivation of the second half (Wehr and Wirz-Justice, 1982; Wehr et al, 1983).

Phase advance of the sleep-wake cycle has been found very effective in several case studies, causing rapid improvement from depression, lasting for a few months and up to 1 year (Sack, Nurnberger, Rosenthal, Ashburn and Wehr, 1985; Wehr et al, 1983; Wehr and Wirz-Justice, 1982) and is potentially a very useful method.

Considering that the clinical efficacy of the major groups of antidepressant drugs has been connected with their ability to phase-delay circadian rhythms (section 3.6), this phase-advance, which presumably constitutes the antidepressant element of sleep therapies, would look contradictory. However, when applied at the same time, it was found that the phase-advance of the sleep-wake cycle enhanced the antidepressant action of drugs (Sack et al, 1985). On their own, the two treatments can be paralled with a clock; one can set the right time by moving the hands clockwise or anticlockwise until the right time is set. Simultaneous application of the two treatments,

however, apparently results in the potentiation of one of the two ways to set the clock.

4.8. Conclusions

If we combine the data from the clinical findings and the experimental manipulations of the possible abnormal functions, it appears that the depressive process (a) is sleep dependant and (b) requires that "sleep coincide with a sleep-sensitive early morning circadian phase" (Wehr and Wirz-Justice, 1982; Wehr et al, 1983). Together, they form the hypothesis that "depression occurs in susceptible persons when a sleep-sensitive phase of the circadian system becomes advanced from the first hours of waking into the last hours of sleep and interacts with sleep to cause depression" (Wehr and Wirz-Justice, 1982; Wehr and Goodwin, 1983b). According to the present knowledge on the regulation of circadian rhythms, the underlying mechanisms that could produce changes whose expression would accord with the described findings could include (a) a short intrinsic period of the circadian pacemaker, so that, upon entrainment, it would adopt an earlier phase position and somehow lock into it; (b) decreased strength of the oscillator modifying its coupling to the zeitgeber despite a normal intrinsic period; (c) hypersensitivity to the zeitgeber (light) causing a phase-advance, upon exposure to the zeitgeber during a susceptible period like the end of the sleep phase (Wehr and Wirz-Justice, 1982; Wirz-Justice, 1983; Kripke, 1983). The considerable efficacy of sleep and light therapies serves as a reinforcement of these speculations.

Finally, it would be useful to note a few general points that

emerge from these studies.

First, in view of the potential importance of light in mood regulation as a result of daily and seasonal adjustments to the light-dark cycle, the increasing prevalence of artificial light in our daily lives, with intensities considerably lower than natural light, qualifies as a possible source of circadian desynchronization connected with depressive illness.

Secondly, it should not be overlooked that sleep and antidepressants cause phase-advance and phase-delay, respectively. Moreover, although tricyclic antidepressant drugs (TCAs), MAO inhibitors and lithium are all connected with causing phase delay, TCAs and MAOIs can precipitate mania whereas lithium is known for its preventive effect, in this aspect, and can even cause a shift in the phase relationship between rhythms driven by different oscillators (Engelman, Pflug, Klemke and Johnsson, 1983). Taken as a whole, these points strengthen the notion that, in depression, more than one oscillator is affected by differential coupling from a zeitgeber and/or altered phase-relationship between them. Therapies, pharmacological and "environmental" (sleep and light therapies), induce their effects by relative re-entrainment of the oscillators. The fact that neither all depressive patients exhibit the same symptoms of circadian asynchrony nor do all treatments result in a positive effect may be due to two factors.

(a) depressive patients may be classified into subgroups, considering that mood variation, REM sleep and temperature rhythms have been shown to distinguish different classes of patients. Phase-advance, as a sole characteristic of circadian abnormalities can lead

to overlooking variations in symptomatology.

(b) Drug therapies are not as yet based on the phase of the illness. Since antidepressant treatments as a whole appear to affect circadian organization it should be expected that the time of drug administration should depend on the phase of the circadian rhythm, as determined by physiological and laboratory parameters. Morever, according to the argument above, the therapeutic efficacy of any one treatment might depend on how the patient is diagnosed in terms of circadian asynchrony, which, in turn, would dictate the kind of therapy and drug administration time.

And, finally, if clinical studies employed the specific methodology developed for identifying and quantifying circadian rhythms, and, conversely, chronobiological studies paid more strict attention to clinical aspects, the benefits from the combined research in the two disciplines might outnumber the existing methodological hazards.

The possibility that affective illness may be partly caused or expressed by abnormalities in circadian rhythmicity, with obvious implications for therapy has not been immune from constructive criticism (Thompson, 1984). Still, the wealth of evidence clearly suggests that it might be rewarding to consider in the future the conventional neurotransmitter theories of depression under the light of the present findings.

CHAPTER 5. ELECTROCONVULSIVE THERAPY (ECT)

5. ELECTROCONVULSIVE THERAPY (ECT)

So far, we have examined the relationship between 5-HT function and depression and also the effects of pharmacological treatment of depression on the 5-HT system. Another antidepressant treatment, which, depending on the clinical picture, can be either the method of choice or an alternative to drug therapy is electroconvulsive therapy (ECT). Its introduction in therapy preceded that of antidepressant drugs and has been marked by much controversy on both scientific and ethical grounds, to the point of being likened, undeservedly, to the use of electricity by military and security forces under totalitarian regimes.

Since the experimental design of this thesis is centered on the effects of electroconvulsive shock in animals (ECS), it seems appropriate to devote the final part of this introduction to the study of the therapeutic and side effects of ECT in clinical practice and the complementary experimental research.

5.1. A Historical Note

The history of the use of electricity in the treatment of illnesses dates from 46 AD when Scribonius Largus used the discharge of a torpedo fish to curb headache and gout (Frazer, 1982; Salzman, 1978). From then until the 20th century, diverse forms like the application of an electric catfish or the holding of a bare electric wire have been used for curative purposes with little more than anecdotal reports testifying to their efficacy.

In 1934, Laszlo Meduna, a Hungarian scientist, reported the cure of a patient with "catatonic stupor" following the administration of a course of camphor injections. He then proceeded to treat more

patients, mainly schizophrenics, with camphor and also pentamethylenetetrazol (Cardiazol). A few years later, in 1938, Professor Ugo Cerletti and his co-workers Bini and Accornero in Rome applied for the first time a series of electric shocks on a patient who went into a grand mal fit (Fink, 1979; 1984; Frazer, 1982). A new era in psychiatric therapy had begun.

Since then the use of electricity for the induction of convulsions, electroconvulsive therapy (ECT), has changed dramatically: the therapeutic application of the treatment has moved away from the schizophrenics, on whom it was first applied; concurrent medication and a number of modifications in its administration have made its use safer and more effective; and, despite a major decrease in its use when the antidepressant drugs evolved, its benefits were re-evaluated as soon as the side-effects and limitations of pharmacological therapies were recognised.

5.2. Clinical Indications and Efficacy

The initial enthusiasm for the therapeutic efficacy of ECT was closely followed by the realization that it was not a panacea for psychiatric illnesses. Today it is fairly well established that only certain kinds of affective disorders will respond to ECT.

Fink (1979) points out that the group which responds better to ECT is that of patients with depressive psychosis (unipolar, bipolar or involutional depression). The same author also supports the use of ECT in mania and acute, but not chronic schizophrenia.

Kendell (1981) reviewed a number of multi-centre and double blind trials, designed to elucidate the therapeutic spectrum and

efficacy of ECT, and concluded that ECT is invaluable in depression and also acute schizophrenia. Chronic schizophrenia and mania, however, are not indicated for ECT.

The same conclusions were reached by Frazer (1982) who also stressed the all too important fact that ECT is irreplacable in depression when the danger of suicide is present, because of its rapid management of this clinical state. It is interesting to note that psycho-motor retardation (and loss of appetite) are possibly among the first symptoms to be alleviated by ECT, before the patients recover completely; increased motor activity without accompanying mood improvement could again increase the risk of suicide (Browning and Cowen, 1986).

On the other side of the spectrum, Small, Small, Milstein, Kellams and Klapper (1985) reported the beneficial effects of ECT on mania. Moreover, they pointed out that manic patients who were not responsive to unilateral ECT benefited from bilateral treatment and implicated the importance of the behavioural state of the patient, as opposed to the strict diagnosis, as a factor determining the choice of electrode placement. An earlier retrospective study had also found ECT effective in the management of mania (McCabe and Norris, 1977).

The superiority of ECT over pharmacological treatments was reviewed in the Royal College of Psychiatrists' memorandum (1977) which sought to establish guidelines for the proper use of ECT. The memorandum acknowledged the higher therapeutic success rate of ECT compared to antidepressant drug treatment in psychotic depression or manic-depressive depression of recent onset, and many other workers have come to the same conclusions (Barton, 1977; Crow and Johnstone,

1979; Crowe, 1984; Janicak, Davis, Gibbons, Ericksen, Chang and Gallagher, 1985; Ottosson, 1985).

Janicak et al (1985) and Gregory, Shawcross and Gill (1985) also provided evidence that ECT is superior to simulated ECT or placebo. Finally, Fink (1979) in his extensive examination of ECT, highlighted the fact that whilst ECT appears to be more effective than drug therapies, the concurrent administration of ECT and antidepressant drugs is, if anything, beneficial. Avery and Winokur (1977) in a retrospective study of a large number of patients also found ECT superior to drug therapies in eliciting improvement and allowing rapid discharge from hospital, but equally successful to drugs or drug-ECT combination in terms of total recovery.

Despite the relative uniformity of the conclusions just presented, most of the authors alert the reader to the problems of evaluating and comparing studies on ECT.

First, ethical considerations have made almost impossible the carrying out of controlled, double-blind trials.

Secondly, there is an enormous variability in the classification of psychiatric patients. The diagnostic criteria for affective illness have changed spectacularly since the early days of ECT and even today psychiatrists of different nationalities tend to evaluate symptoms in different, sometimes opposing ways. To this end, the use of observer and self-assessment scales and classification systems has helped to a certain extent to bridge the gap of diagnostic variability which is dictated by the different psychiatric schools.

Another factor that complicates analysis of clinical effects is the uncontrolled and/or unreported use of previous or concurrent

medication of patients included in a study. Since many drugs are known to exert effects for long periods of time after their discontinuation, insufficient information about their use necessitates the recognition of the possibility that the net effect may be the result of the, synergistic or antagonistic, action of the two treatments.

The much wider use of antidepressant drugs in the last two decades tended to leave only non-responders exposed to ECT treatment. This is naturally a limitation, since the patients who are referred for ECT are not necessarily those who would be treated with ECT if the treatment was considered an equal alternative to drugs by the psychiatrists.

The use of different electrode placements and waveforms in the administration of ECT complicates the situation even more, making inter-study comparisons a hazardous task.

Finally, the scarcity of follow-up studies does not enhance the evaluation of the long-term effects of ECT and the persistence of its side-effects.

In conclusion, ECT is a treatment of choice in conditions like psychotic depression and should be considered as an alternative in the management of acute schizophrenia and, possibly, mania.

5.3. Administration of ECT

The administration of ECT is achieved by a pair of stainless steel electrodes placed on the head of the patient and connected to one of the commercially available ECT machines. The placement of the electrodes can be either bilateral or unilateral on the non-dominant hemisphere (Fink, 1979; Ottosson, 1985). The significance of the

electrode position with respect to production of side-effects will be considered below. In terms of efficacy, both placements produce almost equally successful results (Royal College of Psychiatrists, 1977; Crow and Johnstone, 1979; Kendell, 1981; Crowe, 1984; Rosenberg and Pettinati, 1984; Ottosson, 1985; Janicak et al, 1985; Gregory et al, 1985), although a limited number of studies have shown right unilateral placement to have poor outcome (Steif, Sackeim, Portnoy, Decina and Malitz, 1986).

Also of importance are the type and intensity of the current. The indications are that the use of threshold currents is effective, while exceeding them increases post-ictal confusion and memory disturbances without increase in efficacy (Kendell, 1981). In terms of type of current, sinusoidal wave form currents are as a rule delivered. Brief-pulse stimulus therapy which requires half the electrical energy involved, seems to have the same efficacy (Crowe, 1984; Kendell, 1981). In a study on rabbits, Hyrman, Palmer, Cernic and Jetelina (1985) showed that high-frequency (200 Hz) brief pulse stimuli can induce a brain seizure with minimal energy absorption by the brain.

The ensuing seizure, which is regarded as both the therapeutic ingredient of ECT and the end-point of each session, can be monitored either clinically (motor manifestation of convulsion) or electroencephalographically (brain seizure); there seems to be little doubt as to the usefulness of EEG monitoring in ensuring maximal efficiency with minimal risk of side-effects (Christensen and Koldbaeck, 1982; Pettinati and Nilsen, 1985).

Finally, ECT should be considered and treated as a surgical

procedure. The patient is pretreated with atropine, muscle relaxation is induced by succinylcholine and anaesthesia by methohexitone or thiopentone (Fink, 1979; Frazer, 1982). Continuous oxygenation should always be available since it reduces the risk for side-effects (Fink, 1979). Under these conditions the "modified" ECT bears no resemblance to the "straight" ECT from which it descended.

5.4. Effects of ECT and ECS

5.4.1. Effects of ECT on Humans

5.4.1.1. Effects on Hormonal Systems

The effects of ECT on the hormonal system of man are not generalized; rather, ECT has a selective effect on some of them (Whalley, Dick, Watts, Christie, Rosie, Levy, Sheward and Fink, 1982).

ECT causes a rapid but transient increase in plasma prolactin levels that is seen within 1.5 h after treatment (Linnoila, Litovitz, Scheinin, Chang, and Cutler, 1984a) and is not accompanied by an increase in growth hormone. Prolactin levels can also be elevated by endogenous opioids, but since pretreatment with naloxone does not block prolactin elevation after ECT, the increment is apparently directly attributable to ECT (Papacostas, Stefanis, Markianos and Papadimitriou, 1985). Interestingly, the dopamine metabolite HVA does not follow the prolactin increase in plasma after ECT: this suggests either that prolactin increase is independent of brain dopamine, a hypothesis that contradicts well-founded evidence for the opposite view (Moore, Annunziato and Gudelsky, 1978); or that dopaminergic, hypophyseohypothalamic tract neuron activity contributes only minimally to the overall concentration of HVA in

plasma (Linnoila et al, 1984a). ECT has also been shown to potentiate the 5-HT-mediated response of plasma prolactin to thyrotropin-releasing hormone (TRH), and to leave unaffected the apomorphine-induced suppression of prolactin secretion (Lerer and Sitaram, 1983). Enhancement of the latter response has also been reported (Balldin, Granerus, Lindstedt, Modigh and Walinder, 1982) but only when measured in a mixed population of depressed patients and patients with Parkinson's disease, who responded differently to ECT. It is worth mentioning that prolactin release is enhanced by neuroleptic drugs even at 12 days after discontinuation, an implication that should be considered whenever previous medication has not been withheld from ECT patients used in clinical studies.

Growth hormone (GH) levels do not change following ECS (Linnoila et al, 1984a; Whalley et al, 1982). Apomorphine-induced stimulation of GH secretion was not enhanced by ECT (Balldin et al, 1982) nor was the response of GH to clonidine and methylamphetamine (Slade and Checkley, 1980).

Cortisol response to methylamphetamine was enhanced, following ECT (Slade and Checkley, 1980) but plasma cortisol levels were unchanged 6 minutes after ECT (Whalley et al, 1982) suggesting that perhaps cortisol levels correlate more with clinical outcome rather than direct effects of ECT.

In a thorough investigation of ECT effects on plasma levels of hormones up to 90' after treatment, Whalley, Eagles, Bowler, Bennie, Dick, McGuire and Fink (in press) conclude that ECT massively and rapidly increases levels of prolactin, ACTH, nicotine- and estrogen-stimulated neurophysins, mildly increases luteinizing hormone and

cortisol, decreases GH and leaves TSH unaffected. Their evidence points towards mediation through central serotonergic mechanisms as a mode of action for ECT.

5.4.1.2. Effects on Neurotransmitter Systems

Investigation into the effects of ECT on neurotransmitter function is more difficult in humans. Plasma urine analyses are of limited use and brain tissue is clearly available only in post-mortem studies. That leaves cerebrospinal fluid, sampled in the presence or absence of probenecid, to provide answers on the effects of ECT.

In a group of 6 depressed patients, CSF assayed before ECT and upon recovery showed no changes in 5-HIAA and tryptophan levels despite a slight upward trend (Abrams, Essman, Taylor and Fink, 1976). In a similar study but with the use of probenecid, ECT did not produce any changes in 5-HIAA levels in CSF of patients (Jori, Dolfini, Casati and Argenta, 1975). Negative results were also obtained from assays for 5-HIAA in plasma before and after treatment (Linnoila et al, 1984a). On the contrary, Essman (1978b) reported a decrease in 5-HIAA and increase in tryptophan levels in CSF within 4h after completion of treatment, with 5-HIAA increasing and remaining high for 7 days after.

Studies on the dopamine metabolite HVA showed no changes in CSF (Abrams et al, 1976; Jori et al, 1975) and plasma (Linnoila et al, 1984a) levels following ECT.

The major noradrenaline metabolite MHPG was only slightly increased in plasma (Linnoila et al, 1984a). MHPG concentration in urine of depressed patients was reported low, increased after treatment but did not reach normal levels nor did it correlate with

therapeutic outcome (Joseph et al, 1985). Plasma noradrenaline consistently but insignificantly fell after a course of ECT in depressed patients (Cooper, Kelly and King, 1985) whereas in a (single) case report noradrenaline and adrenaline showed a tremendous increase in plasma from unusually low pre-treatment levels (Khan, Nies, Johnson and Becker, 1985).

Finally, plasma β-endorphin levels showed a transient increase after ECT, which was not sustained during treatment (Misiaszek, Cork, Hameroff, Finley and Weiss, 1984). The increase represents a consistent finding.

As an overall conclusion, it appears that ECT has a fairly consistent effect on the endocrine systems. However, its effect on neurotransmitter systems are variable and controversial and one can, not without danger, speculate that, taken as a whole, ECT may enhance responses depending on 5-HT and NA neurotransmission.

5.4.2. Effects of ECS on Experimental Animals

In the effort to elucidate the mode of action of ECT, the functions of most major neurotransmitter systems have been examined, usually following repeated ECS. The most important results and a summary of all findings are the targets of this section.

5.4.2.1. Effects on 5-HT Function

Despite the immense interest in serotonergic function in depression and as a mediator of ECT's mode of action, little work has been directed towards the "kinetics" of 5-HT. A study on the effects of 1 or 2 ECS, spaced within 30 minutes, showed an increase in

tryptophan and 5-HIAA in rat brain (Tagliamonte, Tagliamonte, di Chiara, Gessa and Gessa, 1972). This work is overshadowed by the often quoted work of Modigh (1976) who reported that 5-HT synthesis and turnover were unchanged after repeated ECS. Recently, 5-HT uptake and release in rat cortex was found decreased, in contrast to both reports (Minchin, Williams, Bowdler and Green 1983).

Binding studies revealed that chronic treatment with ECS does not change [^{3}H]-LSD binding in rat cortex (Cross, Deakin, Lofthouse, Longden, Owen and Poulter, 1979); however, studies with [^{3}H]-spiperone consistently showed increased 5-HT_2 binding sites in rat cortex, following ECS (Green, Johnson and Nimgaonkar, 1983b; Green, Heal, Johnson, Laurence and Nimgaonkar, 1983a; Kellar and Bergstrom, 1983), and enhanced head-twitch response to 5-HTP (Goodwin et al, 1984; Green et al, 1983a, Metz and Heal, 1986). The head-twitch response is attributed to activation of 5-HT_2 receptors (Goodwin and Green, 1985) and the increase in the number of 5-HT_2 sites is seen after 10 daily ECS and 5 ECS over 10 days but not after a single treatment (Green et al, 1983b).

Chronic administration of antidepressant drugs like desmethylimipramine (DMI), zimelidine and mianserin decreased both head-twitch response in mice and 5-HT_2 binding sites in rat and mouse cortex (Goodwin et al, 1984, Metz and Heal, 1986), although an early study reported the opposite effect for DMI (Green et al, 1983a). Evidently this effect of chronic drug administration produced results opposite to the ones taken after chronic ECS, implying that the common therapeutic effect of the two treatments is not mediated necessarily by changes in the postsynaptic regulation of 5-HT.

A report on the firing rate of hippocampal pyramidal neurons in

the rat seems to be in accordance with this conclusion: following ECS, the effectiveness of 5-HT and 5-MeODMT to suppress firing rate was enhanced whereas the response to NA and GABA was not affected.

5.4.2.2. Effects on Catecholamines

Since the catecholamines were the first to be implicated in the cause of depression, a lot of work has been devoted to the effects of ECS on both NA and DA function.

In the same work of Modigh (1976) referred to above, it is shown that whilst DA activity is unchanged, NA activity is increased after repeated ECS. In contrast, brain levels of NA and DA were not changed but HVA was increased, in another work (Papeschi, Randrup and Munkvad, 1974b). An increase in NA uptake and no change in release was reported also by Minchin et al (1983).

Apomorphine, a direct dopamine agonist, causes hypoactivity at low doses and hyperactivity at high doses, the effects being characterized as presynaptic and postsynaptic, respectively. Chronic ECS increased the locomotor activity response to apomorphine and the combination of apomorphine and clonidine in reserpinised mice, a finding that pointed towards increased sensitivity of postsynaptic catecholaminergic receptors (Modigh, 1975). ECS treatment abolished the sedative response to clonidine (Passareli and Scotti de Carolis, 1983) and also depressed the behavioural effects of d-amphetamine and increased the degree of catalepsy produced by reserpine or a-methyl-p-tyrosine (Papeschi, Randrup and Lal, 1974a).

Chronic treatment with ECS or tricyclic antidepressants like DMI down-regulates β-adrenergic receptors (Abel, Clody, Wennogle and

Meyerson, 1985; Birmaher, Lerer and Belmaker, 1982; Nimgaonkar, Goodwin, Davies and Green, 1985). Interestingly, this decrease in the number of β-adrenergic receptors, which can also be caused by a β-agonist like clenbuterol, was prevented by 5,7-dihydroxytryptamine lesions but not pCPA: this indicated that 5-HT neurons, but not 5-HT itself, are needed to elicit the receptor down-regulation (Nimgaonkar et al, 1985). This ECS-induced effect is independent of sex, selectively appears in cortex and hippocampus but not striatum or cerebellum and is not related to the density of any subpopulation of adrenergic receptors in those areas (Biegon and Israeli, 1986; Kellar and Bergstrom, 1983).

As mentioned above, ECS enhanced the locomotor response to high doses of apomorphine (Green and Mountford, 1985; Green et al, 1983a; Modigh, 1975) and prevented the sedative effect of low doses of this drug (Serra, Argiolas, Fadda, Melis and Gessa, 1981). Apomorphine also inhibits DA synthesis; both repeated ECS and haloperidol significantly increased this effect but only the latter agent increased the number of [^{3}H]-spiperone binding sites in rat striatum (Reches, Wagner, Barkai, Jackson, Yablonskaya-Alter and Fahn, 1984).

From these results, it seems that there is not much evidence to link DA function to ECS in alleviating depressive symptoms. In contrast, a down-regulation of β-adrenergic receptors and an increased turnover of NA are two findings which are causally connected and consistently reported, following repeated ECS.

5.4.2.3. Effects on GABA

The most important reason for taking interest in this compound is the evidence that GABA is involved in elevating seizure thresholds (Essman, 1978b) and also the fact that GABA functions are enhanced by benzodiazepines, which are very frequently part of the medication of ECT patients, even during their ECT course.

Repeated ECS did not affect [^{3}H]-GABA or [^{3}H]-diazepam in rat cerebral cortex (Cross et al, 1979). Progabide, a GABA-mimetic drug, enhanced the head-twitch response and increased the number of 5-HT_2 binding sites like ECS, but unlike it, progabide did not decrease β-receptor binding (Green, Johnson, Mountford and Nimgaonkar, 1985). It is interesting that benzodiazepines had the same effect with progabide but when diazepam was injected 5' before or after ECS it abolished the head-twitch response (Green and Mountford, 1985), possibly through an action on the benzodiazepine receptors. Behaviourally, the effects of ECS are different from benzodiazepines: ECS is devoid of anxiolytic action (File and Green, 1984).

5.4.3. Conclusions

There are three strategies that one can use in the examination of the effects of ECS on the various neurotransmitter systems. First, one may attempt to evaluate changes of synthesis, release, metabolism and uptake of the neurotransmitters. Secondly, attention is drawn to how ECS may change receptor number and affinity. Finally, behavioural responses to individual agonists or combination of different agents are considered in terms of pre and post-synaptic action. None of the three strategies on its own can offer sufficient evidence; rather, results should be considered in conjunction to each

other.

Of the six compounds, 5-HT, DA, NA, GABA, acetylcholine and met-enkephalin, only NA is shown to have increased utilization (Green, 1980). That might suggest that only NA is implicated in explaining ECS effects.

However, as it has been shown above, ECS is accompanied by up-regulation of 5-HT_2 and down-regulation of β-adrenergic receptors, hence, the 5-HT system may also be involved.

Furthermore, chronic ECS increased the hyperactivity response produced by tryptophan and tranylcypromine, the locomotor response to methamphetamine and apomorphine and also the locomotor response to apomorphine and clonidine (Grahame-Smith, Green and Costain, 1978). These results clearly indicated that the postsynaptic responses to all three compounds, 5-HT, NA, DA, are enhanced.

Moreover, none of these systems acts independently. When rats underwent destruction of the NA system by bilateral lesioning of dorsal and ventral bundles and locus coeruleus with injection of 6-OH-dopamine, the responses of the animals to 5-HT and DA agonists were unaltered; the enhancement of locomotor responses to the same agents following repeated ECS were, however, inhibited (Green and Deakin, 1980). These results show that 5-HT and DA supersensitivity after ECS may be preceded by alterations in the NA system.

Below, a summary of the effects of ECS is given in the form of a list. Apart from the individual references quoted above, the following reviews have been invaluable: Grahame-Smith et al, 1978; Green, 1980; Lerer, 1984; Lerer and Belmaker, 1982; Lerer and Sitaram, 1983.

EFFECTS OF ECS ON VARIOUS PATHWAYS IN THE CNS

ECS	VARIABLE MEASURED	EFFECT
	5-HT	
single	brain synthesis	↑
repeated	brain content and synthesis rate	<—>
repeated	brain uptake	↓
repeated	head-twitch response	↑
repeated	hyperthermic response	↑
repeated	^{3}H-5HT binding	<—>
repeated	^{3}H-LSD binding	<—>
repeated	^{3}H-spiperone binding, cortex	↑
repeated	hyperactivity following 5-MeODMT	↑
repeated	hyperactivity following quipazine	↑
repeated	hyperactivity following L-tryptophan and tranylcypromine	↑
	Dopamine (DA)	
single	synthesis	↑
repeated	synthesis and brain content	<—>
repeated	^{3}H-spiperone binding, striatum	<—>
repeated	^{3}H-spiroperidol binding	<—>
repeated	DA-sensitivity adenylate cyclase	<—>
repeated	hyperactivity to L-dopa+tranylcypromine	↑
repeated	hyperactivity to apomorphine	↑
repeated	hypoactivity to low dose apomorphine	↓
repeated	stereotypy to apomorphine	<—>
repeated	stereotypy to apomorphine	↑
repeated	locomotor activity to di-b-cAMP into nucleus accumbens	↑
	Opiate system	
single	metenkephalin levels	<—>
repeated	metenkephalin in caudate nucleus	↑
repeated	metenkephalin in pons	<—>
repeated	metenkephalin in hypothalamus	<—>
repeated	metenkephalin in limibic system	<—>
repeated	β-endorphin in hypothalamus	<—>
	Noradrenaline (NA)	
repeated	synthesis and utilization	↑
repeated	brain content	<—>
repeated	uptake at nerve ending	↓
repeated	tyrosine hydroxylase activity	↑
repeated	^{3}H-NA uptake in synaptosomes from cortex of reserpinized mice	↑
repeated	^{3}H-DHA binding (β), cortex	↓
repeated	^{3}H-clonidine (α_2), cortex	<—>
repeated	^{3}H-clonidine (α_2), cortex	↓

		EFFECT
repeated	^{3}H-prazosin (α_1)	↑
repeated	^{3}H-WB4101 (α_1)	<—>
repeated	response to clonidine	↓
repeated	clonidine-induced hypothermia	↓
repeated	clonidine-induced decrease in brain MOPEG-SO_4	↓
repeated	locomotor response to clonidine and apomorphine	↑
repeated	sensitivity of NA-sensitive ad. cyclase	↓
	GABA	
repeated	concentration in caudate nucleus and nucleus accumbens	↑
repeated	synthesis in caudate nucleus	↓
repeated	^{3}H-GABA binding, cortex	<—>
	Acetylcholine (Ach)	
single	choline acetyltransferase in striatum, cortex and hippocampus	transient ↑
single	^{3}H-QNB binding, cortex	<—>
repeated	choline acetyltransferase activity	<—>
repeated	high affinity choline uptake	<—>
repeated	^{3}H-QNB binding, cortex	↓
repeated	^{3}-QNB binding, hippocampus	↓
repeated	atropine-induced increase in cortical ^{3}H-QNB binding	↓

↑ : increase or enhancement
↓ : decrease or inhibition
<—> : minimal or no change

5.5. Adverse Effects of ECT

5.5.1. Memory Impairment and ECT

5.5.1.1. Memory and Amnesia: A Brief Overview

It is beyond the scope of this review to analyse in depth the anatomy, neurophysiology and pathology of learning and memory functions; only a brief overview will be given.

The brain structures that are involved in memory processes are traditionally thought to include the hippocampus, fornix and mammilary bodies, although recent clinical and laboratory findings implicate more brain areas, interconnected by one or more neuronal systems (Oakley, 1981; Squire, 1980).

The cholinergic system is probably the best established mediator of memory functions. Scopolamine, which blocks cholinergic receptors, attenuates human memory whereas physostigmine, a cholinesterase inhibitor, can reverse this impairment (Wurtman, 1980). Diminished levels and turnover of catecholamines and 5-HT in brain have also been linked with memory malfunctions, associated with dementia.

Memory disorders and memory loss (amnesia) can be found in patients with Alzheimer's disease, Korsakoff psychosis, herpes simplex encephalitis and are definitely connected with old age. Neuroanatomically and neurophysiologically, research into the causes of memory impairment is hindered by the fact that it is usually a symptom in patients with a pre-existing illness and extensive clinical findings, not easily related to specific symptoms. The lack of standard tests for the measurement of memory functions in neuropsychology only compounds the confusing pattern of knowledge in the field.

However, it is important to point out the various classifications of memory and its defects. Thus, memory can be short- or long-term; semantic or episodic; and associative, representational or abstract. Memory deficits and amnesia can be characterized as material- or modality-specific, and retrograde or anterograde, respectively (Newcombe, 1980; Oakley, 1981). This list is certainly not exhaustive; moreover, the boundaries of each class are not clearly defined, so that there is a certain overlap between classification systems. Finally, more informative definitions will be given wherever any of these terms is encountered again in the following discussion.

5.5.1.2. Biochemical Aspects of Memory

The study of normal human memory by psychological tests cannot tell us much about the neuronal and endocrine mechanisms that underlie its function. Also, as mentioned above, memory defects are present in illnesses which cause extensive brain changes and, finally, research in humans would inevitably follow the one-way direction of alleviating the symptoms. It is for these reasons that experimental designs that can cause measurable memory impairment had to be devised. The same designs could, of course, be used to investigate the beneficial or detrimental effects of various drugs.

One of the hypotheses on memory formation that cannot easily be tested in humans is the importance of nucleic acids and proteins. Uridine monophosphate, an RNA precursor, and uridine delayed the extinction of an optical discrimination response in rats when injected in the hippocampus 30 minutes before or up to one hour after

training (Ott and Matthies, 1973). These results suggest first that the hippocampus is involved in the information storage processes and secondly that the prevention of extinction is brought about by changes in RNA synthesis and, subsequently, protein synthesis. Orotic acid, a precursor of uridine monophosphate, reversed amnesia induced by ECS in the same kind of experimental conditions (Ott and Matthies, 1971). Conversely, cycloheximide, a protein synthesis inhibitor, produced an amnestic effect in a passive avoidance task in mice, which depended on the strength of conditioning in the animal response (Geller, Robustelli and Jarvik, 1970).

The involvement of the cholinergic system is shown in an active avoidance test (Flood, Smith and Cherkin, 1985); drugs which increase cholinergic function like oxotremorine and edrophonium increased retention in this test both when administered alone and in combination with each other. Morever, in a test that is claimed to resemble passive avoidance tasks and which involves the induction of analgesia by brief footshock immediately after testing for the tail-flick response, oxotremorine and physostigmine reinstated the analgesic response at a time when it was found diminished in control animals (Gower and Tricklebank, 1986).

In human experiments, scopolamine in healthy subjects appears to interfere with the storage, but not retrieval, of information (Ghonheim and Mewaldt, 1975), although this effect was not reversed by administration of choline (Mohs and Davis, 1985).

With respect to 5-HT, Rake (1973) found that 5-HTP, injected after training in a passive avoidance test would restore memory, facilitated by repeated administration of p-chlorophenylalanine, to control levels. Surprisingly, it would also restore to control

levels memory impairment brought about from the treatment with reserpine immediately after training. Evidence from Essman (1978a) was more uniform in that increased 5-HT levels impair, whereas decreased levels enhance memory. Evidence has also been found for the positive role of catecholamines in the consolidation of memory in mice (Dismukes and Rake, 1972).

Finally, vasopressin, a pituitary peptide, has been considered of importance in the mechanism of mammalian memory. An enzyme-resistant analogue of vasopressin was shown to improve impaired and unimpaired cognition and also reverse the retrograde amnesia after ECT (Sahgal, 1984). It is, however, debatable whether the primary effect of vasopressin is on the memory functions or on the behaviour by peripheral and central modulation.

5.5.1.3. Memory Impairment following ECS in Experimental Animals

The administration of ECS for induction of memory impairment has long been used as a model for amnesia and also in an effort to elucidate the causative relation of ECS to amnesia.

Ott and Matthies (1971) showed that when a single ECS followed an optical discrimination test in rats 1 minute to 3 hours after training, it causes retrograde amnesia when tested 24 hours later.

A single ECS also caused retrograde amnesia in a passive avoidance response in mice (Geller et al, 1970). This respone was almost independent of the delay between response and punishment during training. Induction of retrograde amnesia was also shown in a passive avoidance test by Brown, Sissman, Kasprow and Miller (1985) while the effects of ECS were extended to anterograde amnesia in the

same kind of behavioural task (Lerer, Stanley, Keegan and Altman, 1986).

In contrast to Ott and Mathies (1971), Essman (1978d) found that ECS caused retrograde amnesia only when administered between 10" and 10 minutes after training. At 20' the effect is markedly decreased and virtually disappears after 1 hour. Significantly, he also reported that a single ECS inhibited protein synthesis in specific brain areas for at least 5' after treatment, an effect undetectable at 1 hour post-treatment. These effects of ECS on memory and cerebral protein synthesis were blocked by intracranial injection of noradrenaline and normetanephrine, a finding which is very difficult to interpret (Essman, 1978d).

5.5.1.4. ECT and Memory in Depressed Patients

Ever since the treatment became available and throughout its various modifications, reports have been coming in of patients who complained of amnesia of various forms and degrees. Just how important this amnesia is and how, if at all, it can be diminished or avoided is the objective of this section.

ECT is indeed associated with memory impairment. A study of 43 patients with depressive symptoms, treated with bilateral ECT, disclosed that the treatment affected memory for events that took place before the initiation of therapy (Squire, Slater and Miller, 1981). This retrograde amnesia was shown to possess a temporal gradient: events that took place within 1-2 weeks before initiation of treatment seem to be more prone to eradication from memory. Events within 1-2 years before treatment are less affected and memory for events in the remote past of the patients is apparently unscathed

(Squire et al, 1981). Impairment for similar autobiographical memory (memory involving life events of varying degrees and nature) for events close to the admission date was also reported by Rosenberg and Pettinati (1984) for depressed patients and could be related to postictal EEG suppression (Daniel, Crovitz, Weiner, Schwartzwelder and Khan, 1985). As a rule, during a seizure, patients' EEG goes through an initial latency period to a phase characterized by a rapid build-up of diffuse bilateral synchronous spikes and followed by paroxysmal high voltage slow waves, also bilateral and diffuse (Frazer, 1982). This last phase, which coincides with the clonic phase of the convulsion is succeeded by a period of complete EEG suppression with an abrupt onset or by a gradual onset of EEG waves of decreaseed amplitude and increased frequencies (Frazer, 1982). When the suppression is complete, it is shown to correlate with absence of autobiographical memory (Daniel et al, 1985).

Anterograde amnesia, that is, amnesia for events or learning happening after ECT, is also caused by ECT and affects both acquisition and retention of information, as tested by immediate and delayed memory tests, respectively (Steif et al, 1986). This amnesia was transient with respect to immediate memory but delayed memory impairment persisted for 4-5 days. This report could be questioned since the authors clearly state that their tests were performed in all patients, regardless of clinical outcome which was successful in less than 50% of them. The problem that arises is that the non-responders in the study might have contributed to the results in a negative way since it is known that depressed patients complain of impaired memory (Sternberg and Jarvik, 1976). However, most of the

evidence like the one presented by Steif et al (1986), supports the belief that, whilst depression affects the learning processes without diminishing the capacity to retain and reproduce the acquired information, ECT appears to interfere negatively with this retention and retrieval capacity rather than the acquisition phase. With the correct choice of memory tests it is demonstrable that memory complaints due to ECT and those due to depression are of a different nature (Pettinati and Rosenberg, 1984).

With respect to the factors that can influence the effect of ECT on memory, one has first to consider the electrode placement. Unilateral placement was introduced in 1957, in an effort to reduce memory complaints following ECT treatment (Taylor, Tompkins, Demers and Anderson, 1982). Since the left hemisphere is associated with verbal tasks and the right one with visual tasks while motor learning is not linked specifically to either (Newcombe, 1980), a rough estimation of hemisphere dominance dictates the electrode placement on the opposite side of the one that controls verbal skills.

Rosenberg and Pettinati (1984) found more complaints in patients assigned to bilateral rather than unilateral ECT. This finding is fairly consistent and well documented (Fromm-Auch, 1982; Pettinati and Rosenberg, 1984; Squire and Zouzounis, 1986; Weiner, Rogers, Davidson and Miller, 1982).

The choice of wave form has also been implicated as a possible target for eliminating memory complaints. Weiner et al (1982) reported a trend for pulse stimuli to cause less impairment. However, Daniel et al (1985) found no significant difference between brief pulse and sinusoidal ECT on autobiographical memory. In line with this report, Warren and Groome (1984) had earlier shown that

neither wave form could cause memory complaints; indeed, a complete improvement of impaired, pre-treatment memory functions was observed which paralleled the clinical improvement of the patients. It seems that the lack of difference in outcome between the two wave forms as to their action on memory is due to difficulties in administering brief-pulse ECT at low energy levels without jeopardizing its therapeutic efficacy (Squire and Zouzounis, 1986), although the energy level should not be overestimated as a modifiable target (Warren and Groome, 1984).

A final factor to be considered is seizure duration. This has been shown to affect retrograde and anterograde amnesia for non-verbal, but not verbal, material, without correlating with the clinical outcome. These two findings together suggested that, possibly above a minimum seizure duration, therapeutic effect is always achieved whilst the danger of amnesia increases with increase in seizure duration (Miller, Faber, Hatch and Alexander, 1985).

It is obvious from these reports that there is a body of evidence which indicates that ECT produces amnesia of a form that is distinguishable from depression-induced memory impairments and that is possibly controllable by altering ECT parameters, like electrode placement, stimulus wave form and seizure duration. Taylor et al (1982), reviewed 39 publications covering 40 years, on the problem of memory complaints following ECT. The difficulties they faced in interpreting those reports stemmed from a number of factors that varied immensely among the reports. Below, there is a list of these variables, comprised mainly of the ones mentioned by Taylor et al (1982), Fink (1979) and also some points which evolved during the present review.

- Studies on memory functions following ECT are mostly not blind. Some of them do not include a control group or the controls are other psychiatric patients with unknown memory problems.

- Some studies are single case reports.

- The number of treatments, electrode placement, wave form and duration are either not reported or vary considerably between studies.

- The assessment of seizure induction and therapeutic outcome are frequently missing.

- Age as an influencing factor is not adequately considered.

- The diagnosis and severity of illness cover a very broad spectrum.

Testing for memory is equally characterized by lack of uniformity and sensitivity.

- There is a variability in the extent of memory intactness prior to ECT.

- The time of original exposure to information, the timing of the recall test within the treatment and the time of testing after treatment are not standardized.

- The interval to follow-up studies can considerably affect memory performance.

- The modality tested can be auditory, tactile or visual.

- The material can be verbal or figural.

- The procedure may require recalling, relearning or recognition of information.

- Educational and occupational backgrounds introduce another variable depending on the nature of the test.

- The widely advertized problem of amnesia, to which the prospective ECT patient is anyway alerted, introduces a bias towards recording many otherwise unnoticeable memory difficulties.

Finally, there is a complication that does not appear to have been evaluated so far. A sound experimental protocol would require that follow-up studies be carried out at a predetermined time interval, common to all patients. However, the intrinsically rhythmical nature of the illness, the lack of techniques in evaluating the precise phase of the illness, the probable variability both in time required to achieve therapeutic effects and in the degree of recovery following a set number of treatments imply that perhaps a follow up study would lead to erroneous results, if conducted at a common predetermined time interval. If, instead, the studies are conducted at intervals dictated by the degree of clinical recovery of each patient, confounding factors such as postictal confusion, inadequate treatment and illness relapse, will be

effectively eliminated. The results will, therefore, mirror more convincingly the real effects of ECT on memory of patients who have responded to the treatment.

In spite of all the problems analyzed above, Taylor et al (1982) concluded that:

- A standard treatment with bilateral ECT induces memory impairement.
- An increase in the number of treatments results in cumulative effects of memory changes.
- The majority of these changes are reversible with a return to pretreatment levels or better within 6-7 months.
- Some subtle defects usually related to autobiographical material may persist but tend to be of an irritating rather than incapacitating nature.

To these it should be added that:

- Memory complaints ought and may be distinguished from amnesia caused by a course of ECT.
- Unilateral electrode placement eliminates memory impairment or minimizes its duration and severity.
- The frequency of ECT administration, wave form choice and seizure duration can all contribute, in varying degrees, to the presence and extent of amnesia.

It is here appropriate, even if slightly ironic, to point out the value of ECS-induced memory impairment in research for drugs against amnesia of any origin (Butler, Nordin, L'Italien, Zweisler, Poschel and Marriott, 1984; Hock and McGaugh, 1985).

5.5.2. Other Side-effects, and Contra-indications, of ECT

Apart from amnesia there are only a few other major complications of ECT, which are nevertheless very rare: organic psychosis, spontaneous (tardive) seizures, fractures and death. As for minor risks these include postseizure headache and confusion, nausea and skin irritation at the site of electrode placement. Proper ECT administration effectively eliminates practically all these undesirable effects (Fink, 1979).

ECT elevates heart rate, after an initial bradycardia, which lasts 4-6 min. This rise causes a massive, though transient, increase in cerebral blood flow and intracranial pressure (Hamilton, Stocker and Spencer, 1979). For these reasons ECT is not indicated in patients with brain tumors, demyelinating disease (multiple sclerosis) and cerebral aneurysm or cardiovascular diseases like cardiac arrhythmia, recent myocardial infarction and hypertention (Frazer, 1982; Salzman, 1978).

AIM OF THE STUDY

If the reader has patiently read through the extensive introduction he may have already appreciated the necessity of examining in such detail four major areas from the disciplines of psychopharmacology, chronobiology and psychiatry.

The rationale was to reach the following important conclusions: (a) Depression is an illness which may be preceded or followed by abnormal function of the 5-HT system. Prevailing among the symptoms of depression are abnormalities that could be due to impairment of the circadian system. An effective treatment for certain types of depression is ECT. (b) Aspects of 5-HT function are characterized by circadian rhythmicity. ECT may influence serotonergic activity.

The question that arose was whether the therapeutic effect of ECT could be related to interference with the circadian system and especially the circadian rhythms in 5-HT function or 5-HT-associated behaviours.

For this purpose, the concentration of tryptophan and 5-HIAA has been determined in rat CSF over 24 hours and also following acute or chronic administration of ECS. The usefulness of CSF and the use of probenecid in evaluating 5-HT function were further examined following acute administration of a variety of pharmacological agents.

The circadian rhythm of locomotor activity of mice and rats subjected to repeated ECS under conditions of a normal light-dark cycle and constant light or constant dark has been measured. Results were also obtained following chronic antidepressant drug administration in constant light.

The effects of acute or chronic ECS on memory have also been

examined, in a passive avoidance response test in mice, while varying both the sequence of learning the response and ECS treatment, and the interval between them.

Finally the effects of ECS on postsynaptic 5-HT receptors were studied using the head-twitch response in mice and compared to the effect of two antidepressant drugs.

In summary, the purpose of these experiments was to examine the effects of ECS on the circadian rhythm of 5-HT neuron function and behavioural indices, in which 5-HT is implicated.

CHAPTER 6 MATERIALS AND METHODS

6. MATERIALS AND METHODS

In this chapter a detailed analysis is presented of the methods that have been used throughout these studies. Any modifications, as well as additional methodology, are cited in the appropriate place in the following four chapters.

6.1. Animals

Male WISTAR rats (University of Bath strain) were maintained in the animal unit in groups of 8-10, under a 12-hour light - 12-hour dark schedule (lights on at 7.00 am). All animals had free access to standard rat chow (Labsure CRM diet) and tap water.

Male and female CFLP mice (University of Bath strain) were maintained in a separate section of the same unit in groups of 20-25, under a 14-hour light - 10-hour dark schedule (lights on at 5.00 am). All mice were allowed food (Labsure CRM diet) and tap water ad libitum.

Both rats and mice were removed from the stock rooms, transferred to the experimental room, and randomly allocated into groups, at least 48 hours before the commencement of any experimental procedure. The age and weight of the animals varied according to the nature of the experiment but also to availability. In general, the age at the beginning of an experiment ranged from 4 to 10 weeks. All experiments were designed to terminate before the animals were 12 to 14 weeks old. More information is provided at the beginning of each subsequent chapter.

6.2. Light Conditions

In many cases, there was a need to alter the light-dark

conditions under which the animals were kept. Since these changes are extremely important for this work, it is appropriate to introduce the abbreviations that will be used in the text and figure legends.

Examples: L:D 14:10; a normal 14 hour light - 10 hour dark cycle, lights on at 5.00 am (mice).

D:L 12:12; a reversed cycle where the dark and light phases are of equal length to the initial ones but the time that lights come on is different from 7.00 am (rats).

L:L; constant light conditions.

D:D; constant dark conditions.

The reversed or constant conditions were achieved either by placing the animals in an experimental room with controllable light switch or by the use of specially built cabinets.

6.3. Environmental Cabinets

These environmental cabinets have been described by Hillier, Davies and Redfern (1973). They consist of 17 mm blockboard and measure 45.5 x 61 x 59 cm (fig 6.1). The interior of each cabinet and the inner surface of its door are coated with 12 mm polystyrene sheets. To achieve maximum sound and light insulation, a 13 mm commercial draft excluder tape is placed between the door of each cabinet and the cabinet walls. A 30 mm-bore rubber tube connects the back of each cabinet with an extractor fan (Philips H3 3408), which catered for the ventilation of the cabinets and the maintenance of temperature in the interior at the same level with the experimental

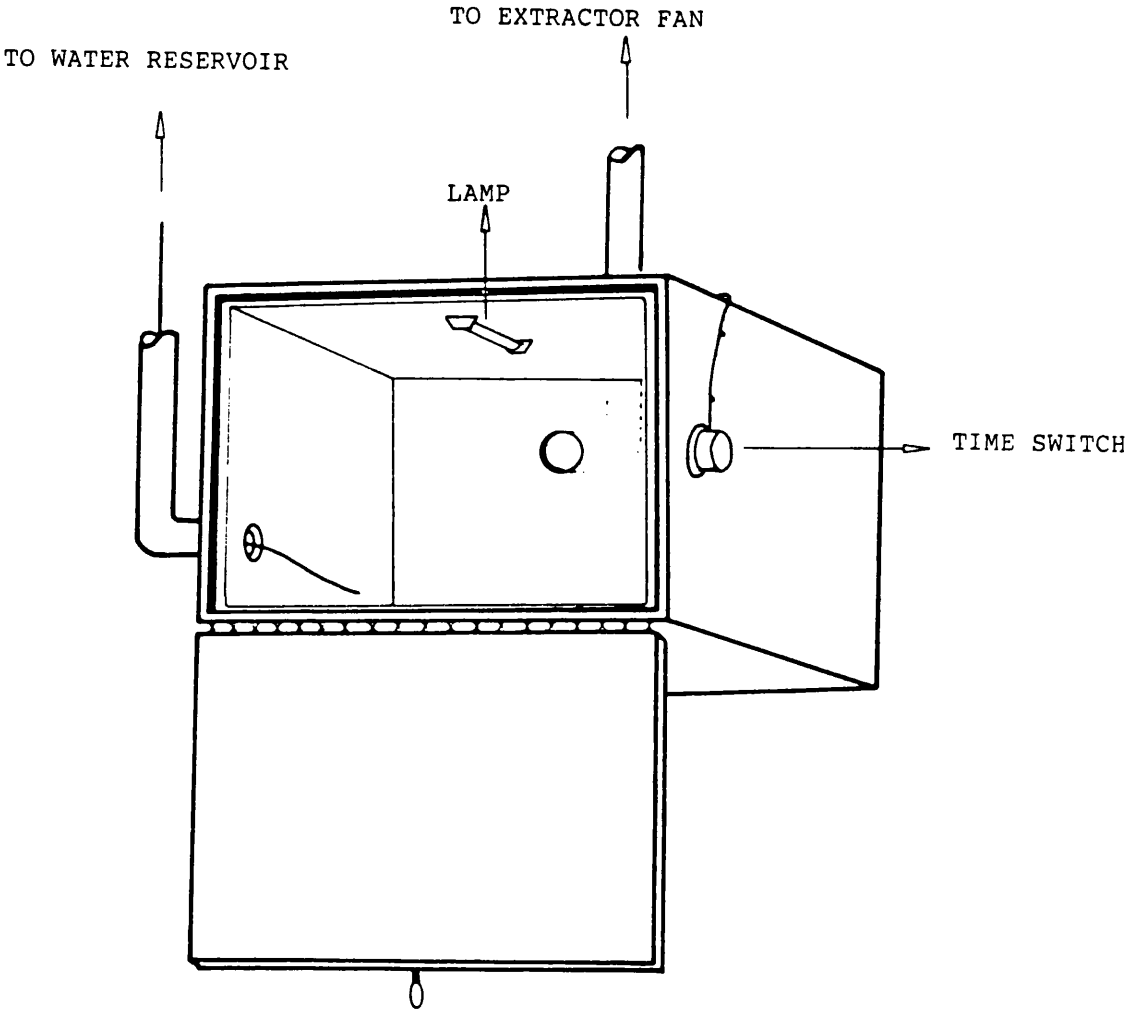


Figure 6.1 Diagram showing the main features of an environmental cabinet.

room, i.e. 23 ± 2°C. A 12", 8 Watt white fluorescent light bulb (Osram) is fitted in the roof of each cabinet, with the choke (Thorn) placed on a metal platform on the outside, to prevent excessive heating of the interior. A time switch (Sangamo, Type 5254-I-171) enables the independent control of the light conditions in each pair of cabinets. Finally, a water reservoir above the cabinets is connected to each cabinet with a flexible water pipe system, running on the outside of the cabinets and entering them through a second piece of rubber tubing.

Once in the cabinets, the animals were disturbed only at infrequent intervals, in order to replenish food and provide clean cages, or whenever treatment was required.

6.4. Electroconvulsive Shock (ECS)

A series of pilot experiments was conducted with mice and rats in order to identify the convulsive threshold. ECS was administered at a potential ranging from 80 to 150 V, frequency 40 to 70 Hz, duration of pulse 1 to 10 ms and duration of stimulus 1 to 5 seconds. From these studies, the following settings were chosen.

Mice: 100 V, 50 Hz, 10 ms, for 1-2s

Rats: 150 V, 50-60 Hz, 8-10 ms, for 1-2s

The current was delivered throught a set of crocodile earclips, filled with copper to give a flat surface and a standardized delivery area. The earclips were connected to a Grass S44 stimulator modified to deliver up to 150 mA at 25 Ω. The pulse generated by this stimulator was monitored by a Telequipment D61a oscilloscope and characterized as undirectional, square wave.

The treatment was delivered under halothane/O_2 anaesthesia,

taking care to retain the animals under anaesthesia for the same amount of time and administer the ECS at the same stage of anaesthesia. The end point of ECS administration was a tonic flexion. Upon discontinuation of the stimulus, the tonic phase usually exhibited full hind limb extension followed by the clonic phase, which lasted approximately 20". The animals were then in a state of stupor for about 5 minutes, after which complete recovery was observed.

Control animals were treated exactly as described above, but no shock was delivered.

It is worth noticing that neither ECS nor halothane anaesthesia were responsible for a single death among the animals treated.

Also, it should be added that frequently the seizure threshold would increase. In such a case, the voltage was increased up to 110 V (mice) or the frequency was changed to 60 Hz (rats). These increments in ECS "dosing" were considered necessary in order to meet the more important methodological criterion of successful ECS administration: the induction of a convulsive, rather than subconvulsive, shock.

6.5. Drugs and Chemicals

Below is a list with all the drugs and chemicals used during the studies, including their source and, where applicable, the vehicle in which they were dissolved.

COMPOUND	VEHICLE	SOURCE
Acetic acid		Fisons Ltd
Carbidopa*	saline	MSD Ltd
Clomipramine*	saline (i.v. admn.) distilled H_2O (oral admn.)	Geigy Pharmaceuticals
p-chlorophenylalanine methyl ester (HCl)	distilled H_2O pH adjusted	Sigma Chemicals
Cysteine HCl	distilled H_2O	Sigma Chemicals
Desmethylimipramine	distilled H_2O	Sigma Chemicals
EDTA		BDH Chemicals
Halothane		May & Baker Ltd
5-hydroxyindole-3-acetic acid		Sigma Chemicals
5-hydroxy-L-tryptophan	distilled H_2O	Sigma Chemicals
Imipramine HCl*	saline (i.v. admn.) distilled H_2O (oral admn.)	Geigy Pharmaceuticals
Lithium carbonate	distilled H_2O	BDH Chemicals
Methanol (HPLC grade)		May & Baker Ltd
5-Methoxy-N,N-dimethyl-tryptamine	saline	Sigma Chemicals
Mianserin HCl*	distilled H_2O	Beecham Research Laboratories
Nomifensine maleate*	ethanol/saline pH adjusted	Hoechst UK Ltd
Paroxetine HCl*	distilled H_2O	Beecham Research laboratories
Probenecid	1.5g dissolved in 5.4 ml NaOH 1M, 9.6ml phosphate buffer (pH7) added, pH adjusted	Sigma Chemicals
Reserpine	250 mg dissolved in 2ml benzyl alcohol, 10 ml Tween 80 added, made up to 100 ml with 30% citric acid.	BDH Chemicals

...contd

COMPOUND	VEHICLE	SOURCE
Sodium Acetate		Fisons Ltd
Sodium Pentobarbitone		May & Baker Ltd
Tranylcypromine sulphate	saline (i.v. admn.) distilled H_2O (oral admn.)	S K & F Ltd
Trifluperidol*	ethanol/water	Janssen Pharmaceutical Ltd
L-tryptophan	distilled H_2O (oral admn.)	Sigma Chemicals

Drugs marked with an asterisk were generously donated by the sources named.

7. THE VARIATION OF TRYPTOPHAN AND 5-HIAA LEVELS IN RAT CSF OVER 24 HOURS AND THE EFFECTS OF ECS AND VARIOUS DRUGS

7.1. AIM

The appearance of tryptophan and 5-HIAA in CSF has been taken in the past as an index of neuronal activity and the arguments for and against such a hypothesis have already been discussed. In view of the evidence pointing towards a circadian regulation of many aspects of 5-HT function, the first objective was to investigate the possibility that the concentration of tryptophan and 5-HIAA in rat CSF might show a circadian variation. The effects of both acute and chronic ECS were then examined during the 24-hour cycle, in search of proof that the mode of action of ECS was related either to changes in the resting levels of the two compounds or in the characteristics of their circadian rhythms, if such rhythms were identified. Finally, aspects of the diagnostic value of CSF and the use of probenecid were also investigated.

7.2. Materials and Methods

7.2.1. Animals

Male WISTAR rats were used throughout. The animals were kept in conditions of L:D 12:12 or D:L 12:12, in groups of 4 to 8 as discussed in the previous chapter. Since the time that lights came on varied up to 12 hours from the normal (7.00 am), the animals were maintained in the new conditions for at least 15 days before they were used. Animals for which no changes in the L:D cycle were necessary were also transferred to the same cabinets for a few days, in order to cancel out any effect of the housing arrangements.

7.2.2. ECS

The practice for inducing anaesthesia and administering ECS were described in detail in section 6.4.

7.2.3. CSF Withdrawal

Sampling of CSF took place under anaesthesia induced by sodium pentobarbitone (Sagatal), 60 mg/kg ip. As soon as anaesthesia was established, each rat was placed on a stereotaxic frame using earbars only. A 1 cm incision was made from the level of the occipital crest downwards. The skin and underlying musculature were retracted, exposing the membrane covering the cisterna magna. A withdrawal cannula was inserted into the cisterna magna at about 30^{o} to the vertical. Each cannula comprised a 2 ml syringe fitted with a 23 G needle and was attached to a 1.5 cm long needle, hand-made out of 1 mm bore metal tube, via a 30 cm length of calibrated polyethylene tubing (800/100/200/100, Portex). CSF was slowly withdrawn to a volume not exceeding 80 μl.

Immediately after withdrawal the collected CSF was transferred into code-numbered Eppendorff tubes and 1 μl of a cysteine HCl 0.01% solution was added for every 10 μl of CSF by a Hamilton syringe (Hutson et al, 1984). The tubes were then placed in a Dewar flask containing solid CO_2 and kept there until they were analysed, routinely within two weeks from sampling date.

7.2.4. High-Performance Liquid Chromatography (HPLC)

Samples of rat CSF were assayed for tryptophan and 5-HIAA on an HPLC system, coupled to an electrochemical detector (HPLC-ED). This consisted of a two-piston pump (LDC III), connected to a 25 cm

column, via a rheodyne valve and a 20 µl loop. The column was packed with Hypersil ODS C18 (5µ). The flow rate was maintained at 1.5 ml/min and the working pressure was approximately 2000 psi.

The mobile phase consisted of 10% methanol (v/v) in double distilled, deionized water (Milli Q, Millipore) containing EDTA (50 µM) and sodium acetate (50 mM), and the pH was adjusted to 4.00 by glacial acetic acid (Curzon, Kantamaneni and Tricklebank, 1981). It was then filtered through a Whatman filter (0.45 µm pore size) in a Sibata (Fuji) filtration system and degassed by helium before use. Any quantities of mobile phase not used within a week from preparation were discarded.

The mobile phase, loop and column were all placed in a water bath and maintained at 31°C by a heating element (Grant Instruments Ltd).

Detection of tryptophan and 5-HIAA was achieved by a set of a working glassy carbon electrode and a Ag/AgCl reference electrode, kept at a potential difference of + 0.80 V. The signal was monitored on an LC-3A detector (BAS) and recorded on a CR 650 S pen recorder (JJ Instruments).

Shortly before assaying, each CSF sample was thawed and filtered through a 5µ filter in a microfilter system (BAS) while being centrifuged at 3000 rpm for 5 minutes.

The assay employed the method of external standardization. Three concentrations of tryptophan and 5-HIAA were made up in Milli Q water and injected in a random order at the beginning of each working day in order to verify the linearity of the response. These injections were repeated at frequent intervals during the assay of

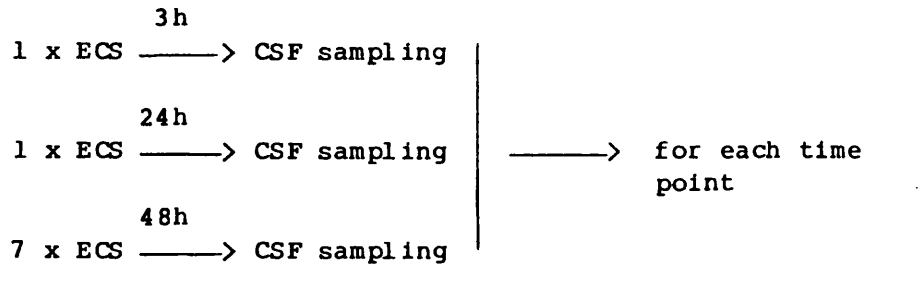
CSF samples to monitor the sensitivity and reproducibility of the method. Fresh standard solutions were prepared weekly. A typical calibration curve for tryptophan and 5-HIAA is depicted in figure 7.21.

The system was frequently flushed with methanol and methanol/water and the pump cleaned with a warm, 30% Decon solution.

7.2.5. Experimental Design

Since, on many occasions, groups of rats were maintained in L:D cycles which differed from the actual clock time, it was considered that experimental time should be calculated on the basis of the time that lights came on for each group. Thus, CSF was sampled at 1,4,8 and 11 hours after lights on, which correspond to four points of the inactive (light) phase and 13, 16, 20 and 23 hours after lights on, a set of four points of the active (dark) phase. In all studies involving only one time point, this was always 4 hours after lights on.

At any given time point, CSF sampling followed either 3 or 24 hours after a single ECS, or 48 hours after the last ECS in a series. Repeated ECS was given every other day, including weekends, up to a total number of seven treatments. Thus, the experimental schedules can be summarized as follows:



Appropriate controls were used in all cases.

Acute drug administration took place 24 hours before CSF sampling, at 4 hours after lights on.

7.2.6. Probenecid Pretreatment

Whenever probenecid was used, 200 mg/kg ip. was injected to the rats one hour before sampling time.

7.2.7. Analysis of Results

The concentrations of tryptophan and 5-HIAA in a sample were calculated from the peak height of the standard solutions and always expressed as the mean $\pm$ sem in nanomoles per millilitre (nmol/ml) of CSF. Groups were compared by Student's t-test. Variation over 24 hours was tested by analysis of variance (ANOVA).

7.3. Results

7.3.1. Effects of Probenecid Pretreatment (I)

The possibility that probenecid administration might be beneficial in examining the effects of ECS on tryptophan and 5-HIAA concentration in CSF without interfering with tryptophan metabolism was first examined. CSF was sampled 5 minutes (fig. 7.1), 3 hours (fig. 7.2) or 24 hours (fig. 7.3) after a single ECS or 48 hours after repeated ECS (fig. 7.4), always 4 hours after lights on.

In each of these experiments four groups of animals were used. Two of these served as controls and the other two were treated with ECS. Moreover, one experimental and one control group were injected with probenecid while the two remaining groups were injected with vehicle.

As shown in figures 7.1-4, there is no detectable effect of ECS on tryptophan, 5-HIAA or their ratio in any of the four experiments.

The effects of probenecid were most prominent on 5-HIAA levels and the [TRY]/[5-HIAA] ratio, increasing the former and decreasing the latter by a factor between 3 and 5. However, the most important point is that probenecid consistently increased tryptophan concentration in both controls and ECS-treated animals, compared to vehicle-treated subjects. Since there was no difference in the tryptophan levels between control and experimental groups treated with vehicle, their mean value was compared to the mean value of control and experimental animals treated with probenecid. Although there was no difference when CSF was sampled within 5 minutes after ECS, tryptophan concentration in probenecid-treated animals was significantly higher than in vehicle-treated animals sampled at 3 hours ($p<0.001$) or 24 hours ($p<0.05$) after 1 x ECS or 48 hours ($p<0.001$) after 7 x ECS. In view of these results, probenecid was not used in the following experiments.

7.3.2. The 24-Hour Variation of Tryptophan and 5-HIAA

When CSF was sampled at 8 different points in a 24-hour cycle it was found that both tryptophan and 5-HIAA concentrations varied significantly with time.

Tryptophan concentrations rose steadily during the dark phase, fell rapidly towards its end and rose again at the beginning of the light phase. Thus, the pattern is characterized by a significant variation (d.f. 7/96, f: 3.22133, $p<0.01$, ANOVA) and two peaks, at 1 and 20 hours after lights on (fig. 7.5).

Similarly, 5-HIAA concentration varied significantly (d.f. 7/96,

f: 9.4662, p< 0.01, ANOVA), also peaking 1 hour after lights on. The pattern of variation resembled that of tryptophan but without a peak at the end of the active phase (fig. 7.5).

The [TRY]/[5-HIAA] ratio, on the other hand, remained fairly constant during the 24 hours (data not shown), the significance of which will be discussed later.

In conclusion, tryptophan and 5-HIAA levels in rat CSF, but not their ratio, displayed a 24-hour variation. It should be added that the results presented here are a compilation of the controls of the experiments that will be described in the following three sections.

7.3.3. Effects of 1 x ECS Given 3 Hours Before CSF Sampling

Treatment with a single ECS, 3 hours before CSF was withdrawn at the 8 set time points did not alter the concentration of either tryptophan or 5-HIAA, or their ratio. Although concentrations in the experimental groups were, as a rule, marginally higher than those of the controls, they only differed significantly on two occasions (fig. 7.6).

The variation of both tryptophan and 5-HIAA concentrations over 24 hours reached significant levels (Tryptophan; Control group: d.f. 7/21, f: 4.42475, p <0.01, ANOVA; Experimental group d.f. 7/19, f: 7.32809, p<0.01, ANOVA. 5-HIAA; Control group: d.f. 7/21, f:13.3686, p<0.01, ANOVA; Experimental group: d.f. 7/15, f: 5.03742, p<0.01, ANOVA) as did their ratio for the experimental group.

The results showed, therefore, that, within 3 hours after treatment, acute ECS does not bring about any changes in the resting levels of tryptophan and 5-HIAA or their variation with time.

7.3.4. Effects of 1 x ECS Given 24 Hours Before CSF Sampling

By extending the sampling interval to 24 hours after a single ECS, the prevailing result was that the diurnal variation of both tryptophan and 5-HIAA disappeared (fig. 7.7) but the result was not exclusive to experimental animals; the control rhythm in tryptophan concentration was also lost and that of 5-HIAA only just reached significant levels (d.f. 7/36, f: 2.71472, $p<0.05$, ANOVA).

No differences were detected in the resting levels of either tryptophan or 5-HIAA between controls and experimental animals at any time point. Consequently, it must be concluded that there is no effect on the examined variables of 1 x ECS administered 24 hours before sampling.

7.3.5. Effects of 7 x ECS

Repeated ECS treatment was given as described above and CSF was sampled 48 hours after the last ECS at each time point. This experimental regime also failed to alter the resting levels of tryptophan and 5-HIAA (fig. 7.8). The variation over 24 hours was again present (tryptophan; Control group: d.f. 7/23, f: 4.02774, $p<0.01$, ANOVA; Experimental group: d.f. 7/31, f: 4.33629, $p<0.01$, ANOVA. 5-HIAA; Control group: d.f. 7/23, f: 4.95811, $p<0.01$, ANOVA) with the exception of the variation in 5-HIAA concentration in the experimental group. The [TRY]/[5-HIAA] ratio between the two groups were comparable and did not display a significant variation.

Although tryptophan concentration for the experimental groups was elevated at 13 and 16 hours after lights on, there was generally no difference in tryptophan or 5-HIAA concentrations between the two groups.

It is, therefore, concluded that repeated ECS is devoid of any notable effect on the concentration of the two substances throughout a 24-hour cycle. Although the rhythm for tryptophan was maintained, [5-HIAA] variation with time fell below significant levels, when ECS was applied.

7.3.6. Effects of Food Deprivation

Groups of rats were food-deprived 24 hours before CSF was sampled. This procedure induced an elevation in tryptophan concentration between 8 and 16 hours after lights on, but no changes at the remaining 4 time points (data not shown). This elevation, during the period that is characterized under normal conditions by low tryptophan levels (fig. 7.5), resulted in the loss of diurnal variation.

The concentrations of 5-HIAA in CSF at all time points but one were greatly elevated in the food-deprived groups (fig. 7.9) and sustained their highly significant variation with time (d.f. 7/29, f: 3.93161, $p<0.01$, ANOVA).

7.3.7. Effects of a Single Dose of Various Pharmacological Agents.

In the following experiments, each drug was administered to two groups of rats whereas vehicle was given to two control groups. The drugs were prepared as described in section 6.5 and injected intraperitoneally 4 hours after lights on. Three hours after lights on, the following day, one drug and one control group were injected with probenecid 200 mg/kg and the remaining two group with vehicle, and CSF was sampled 1 hour later.

The use of probenecid was reintroduced in order to examine its interaction with other drugs and their combined effect on the 5-HT system.

(a) pCPA (150 mg/kg)

A single dose of pCPA presumably inhibited tryptophan hydroxylase and caused an accumulation of tryptophan with a corresponding decrease in 5-HIAA concentration. The [TRY]/[5-HIAA] ratio was also greatly increased. Probenecid pretreatment did not alter these findings (fig. 7.10).

(b) Carbidopa (25 mg/kg)

This relatively low dose of carbidopa did not affect the concentrations of tryptophan and 5-HIAA or their ratio in CSF of rats treated with vehicle (fig. 7.11). Administration of probenecid resulted in higher levels of both substances without affecting their ratio.

(c) Reserpine (5mg/kg)

Administration of reserpine took place 18 hours, rather than 24 hours before sampling at the normal time point of 4 hours after lights on. There was no increase of tryptophan concentration in CSF, regardless of the presence of probenecid (fig. 7.12). The concentration of 5-HIAA was almost doubled and the [TRY]/[5-HIAA] ratio accordingly decreased, a sign that significant 5-HT release must have taken place from the storage pools.

(d) Tranylcypromine (5 mg/kg)

This irreversible, non-selective MAO inhibitor produced a significant decrease in 5-HIAA concentration and a consequent increase in [TRY]/[5-HIAA] ratio without affecting tryptophan concentration (fig. 7.13). Probenecid pretreatment did not modify the results.

(e) Imipramine (20 mg/kg)

The non-selective amine uptake inhibitor, imipramine, did not alter the concentrations of tryptophan and 5-HIAA or their ratio. However, probenecid-treated animals showed increased levels of both substances (fig. 7.14). Since their ratio was also elevated, it could be assumed that the increase of tryptophan was proportionately larger, an effect again attributable to probenecid pretreatment.

(f) Paroxetine (10 mg/kg)

Although paroxetine is considered to have specific 5-HT uptake inhibitor properties, no decrease in 5-HIAA concentration was noted in the presence or absence of probenecid (fig. 7.15).

(g) Nomifensine (5 mg/kg)

The uptake inhibitor properties of nomifensine are more selective for noradrenaline and dopamine and thus no measurable effect would be expected in the present system. Indeed, no changes were seen in CSF concentrations of tryptophan and 5-HIAA (fig. 7.10), an effect independent of the presence of probenecid.

(h) Mianserin (10 mg/kg)

A single dose of mianserin did not change the concentration of 5-HIAA but decreased that of tryptophan (fig. 7.17). This effect disappeared following probenecid pretreatment. The ratio of the two substances was unaffected by the administration of mianserin, irrespective of the presence of probenecid.

(i) Trifluperidol (10 mg/kg)

Acute dosing with the neuroleptic trifluperidol did not alter the resting levels of tryptophan or 5-HIAA or their ratio. In the presence of probenecid, however, the concentrations of both substances were elevated while their ratio was not affected (fig. 7.18).

(j) Lithium (5 mg/kg)

This dose of lithium clealy had no effect on the 5-HT system. Neither the concentrations of tryptophan and 5-HIAA nor their ratio was altered by lithium and pretreatment with probenecid did not reveal any difference between control and drug groups (fig. 7.19).

7.3.8. Effects of Probenecid Pretreament (II)

At the beginning of this section it was shown that probenecid pretreatment might be responsible for an increase of tryptophan concentrations in CSF. Here, all the controls treated with probenecid or vehicle from the experiments described in the previous section (7.3.7) and from a separate experiment were averaged. It was, thus, shown that probenecid pretreatment increased tryptophan levels ($p < 0.01$) as well as causing the anticipated increase in 5-HIAA concentration in CSF ($p < 0.001$) (fig. 7.20). The ratio of the

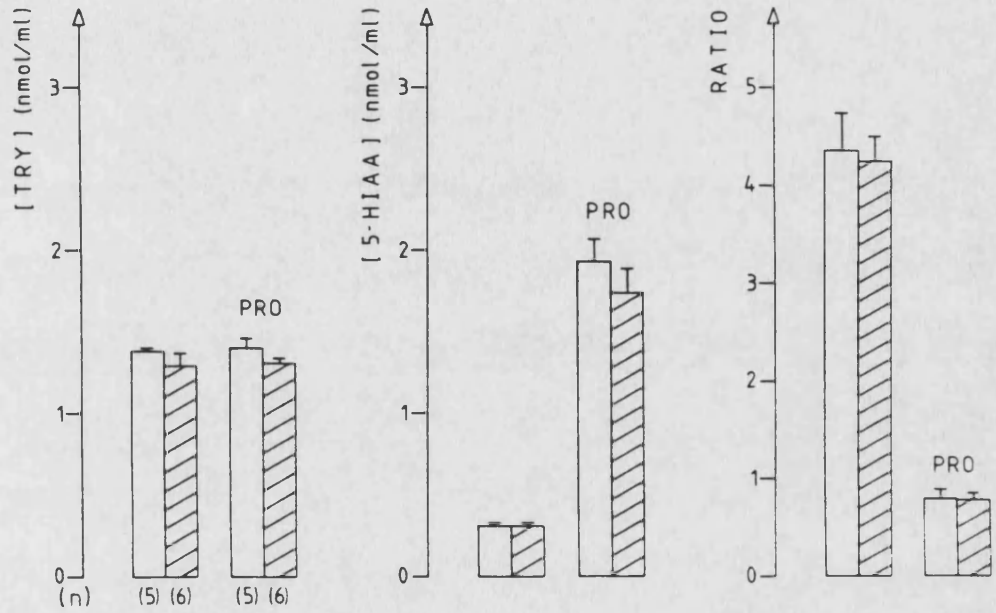


Figure 7.1 The effects of 1 x ECS on tryptophan (TRY) and 5-HIAA concentrations,and their ratio, in rat CSF sampled 5' after treatment. Open columns: control animals. Hatched columns: ECS-treated animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.

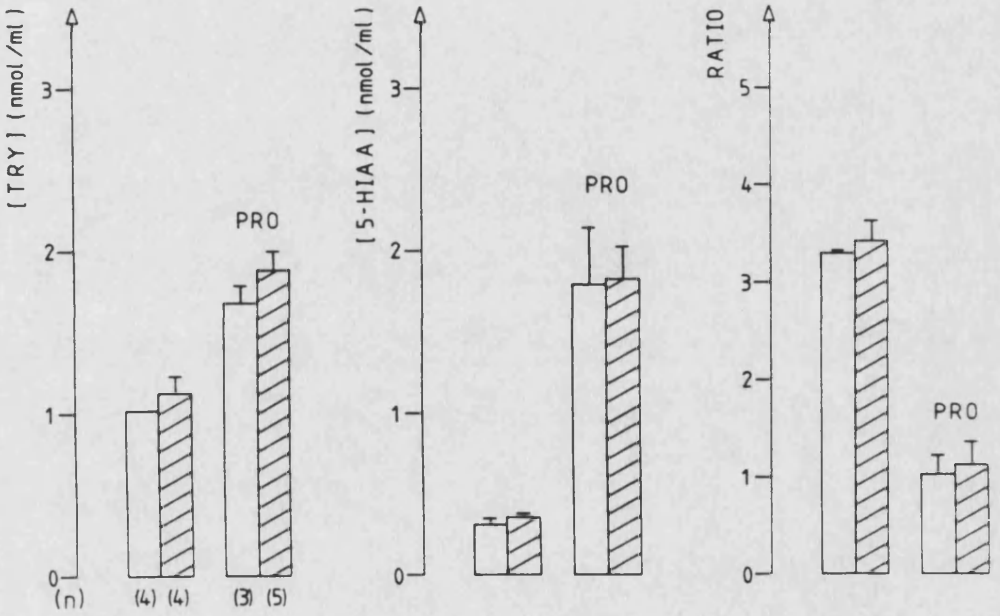


Figure 7.2 The effects of 1 x ECS on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF sampled 3 h after treatment. Open columns: control animals. Hatched columns: ECS-treated animals. Probenecid (PRO) administered 1 h before sampling. Mean $\pm$ s.e.m.

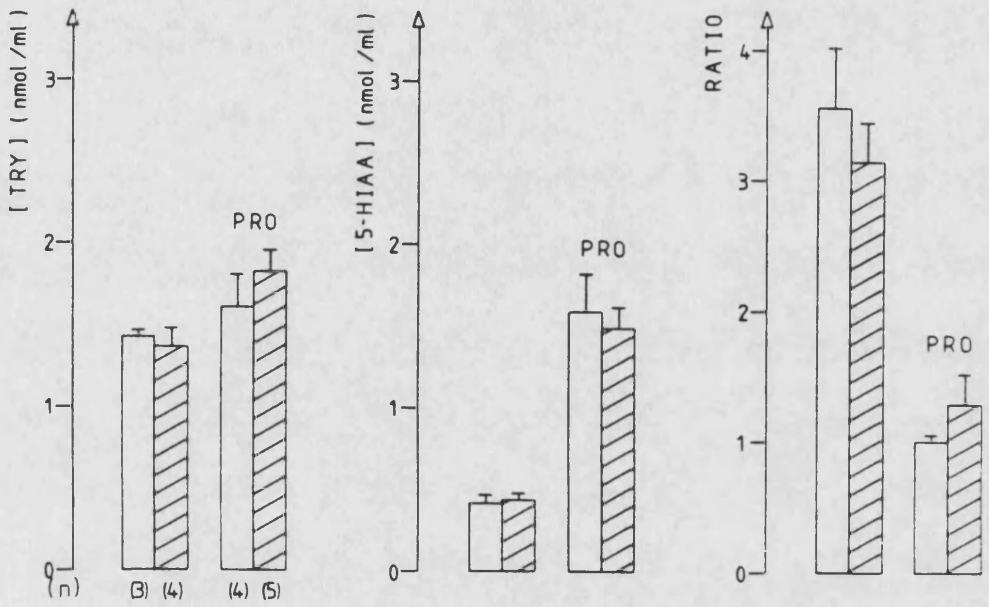


Figure 7.3 The effects of 1 x ECS on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF sampled 24 h after treatment. Open columns: control animals. Hatched columns: ECS-treated animals. Probenecid (PRO) administered 1 h before sampling. Mean $\pm$ s.e.m.

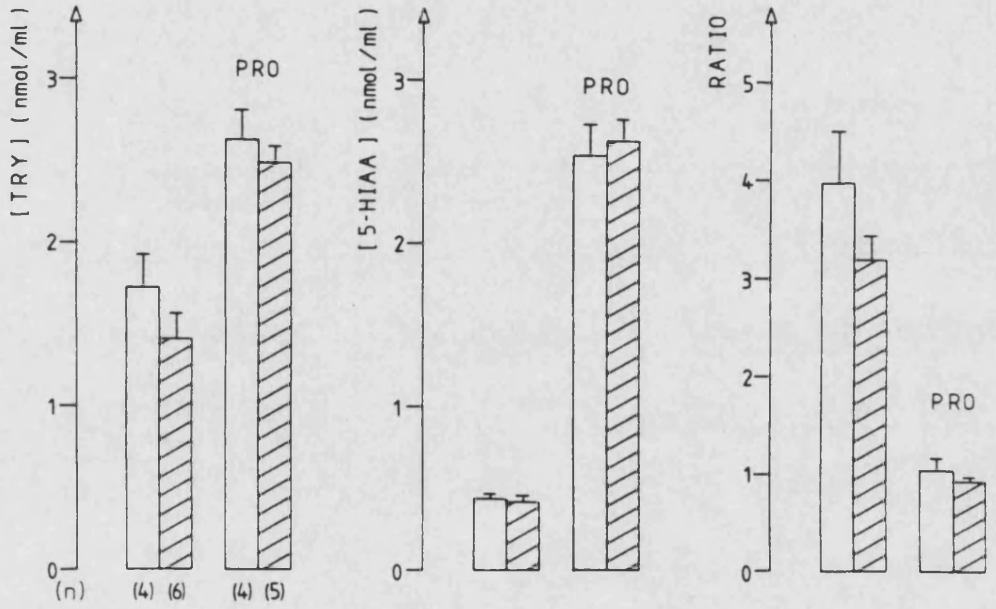


Figure 7.4 The effects of 7 x ECS on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF sampled 48 hr after the last treatment. Open columns: control animals. Hatched columns: ECS-treated animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.

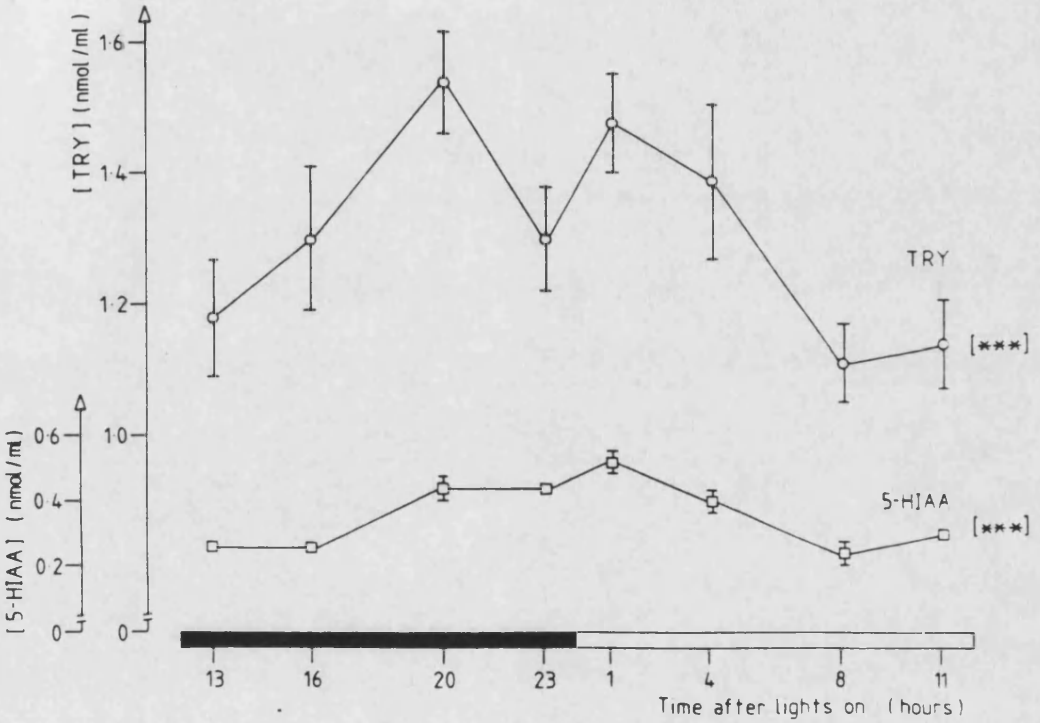


Figure 7.5 Diurnal variation of tryptophan (TRY) and 5-HIAA concentrations in rat CSF over 24 hours. Black bar on time axis: dark phase. Mean ± s.e.m.; n=11-16, at each time point; [***]: $p<0.01$ (ANOVA).

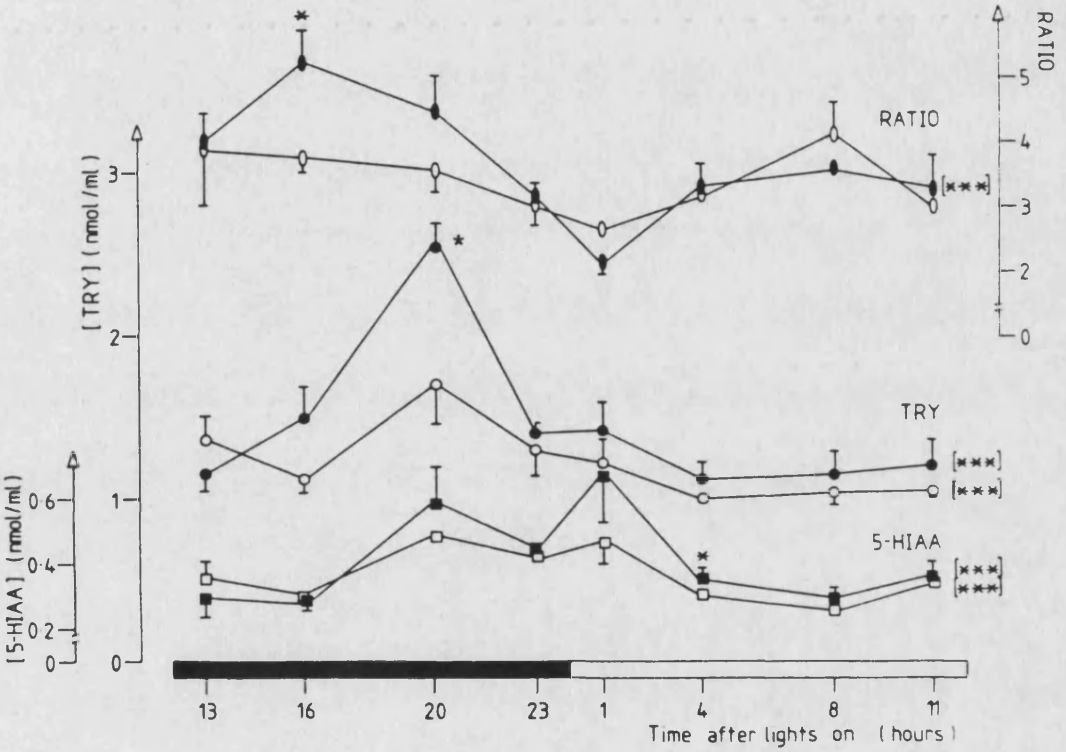


Figure 7.6 Effects of 1 x ECS on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF sampled 3 h after treatment. Open symbols: control animals. Closed symbols: ECS-treated animals. Black bar on time axis: dark phase. Mean ± s.e.m.; *: $p<0.05$ (t-test), compared to appropriate control; [***]: $p<0.01$ (ANOVA).

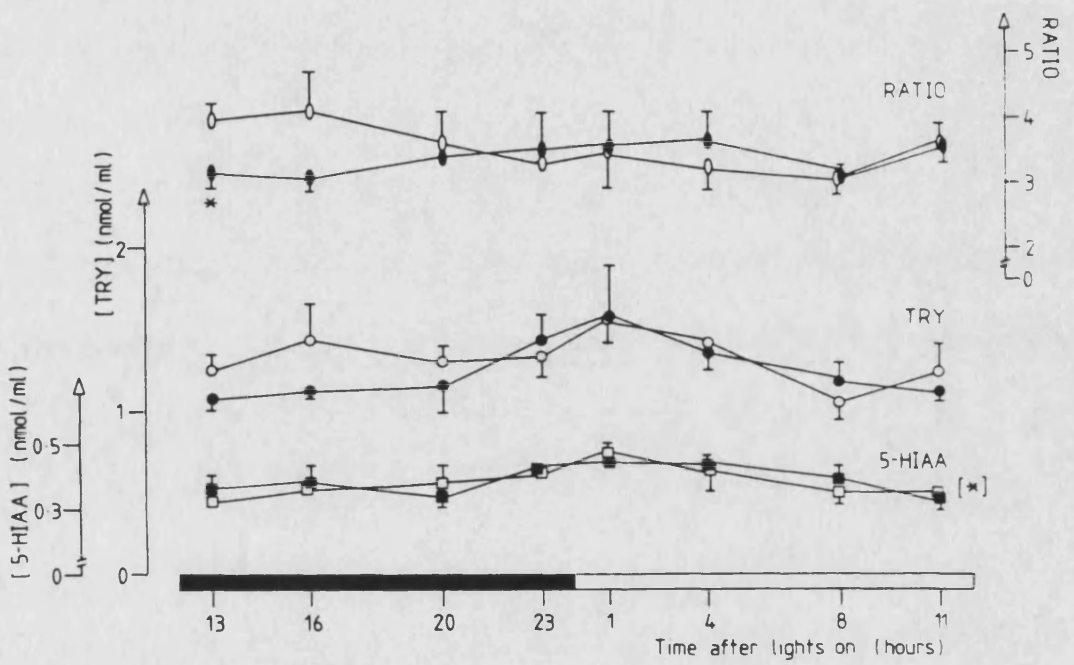


Figure 7.7 Effects of 1 x ECS on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF sampled 24 h after treatment. Open symbols: control animals. Closed symbols: ECS-treated animals. Black bar on time axis: dark phase. Mean ± s.e.m.;

n=3-7, at each time point; * $p<0.05$ (t-test); [*]: $p<0.05$ (ANOVA).

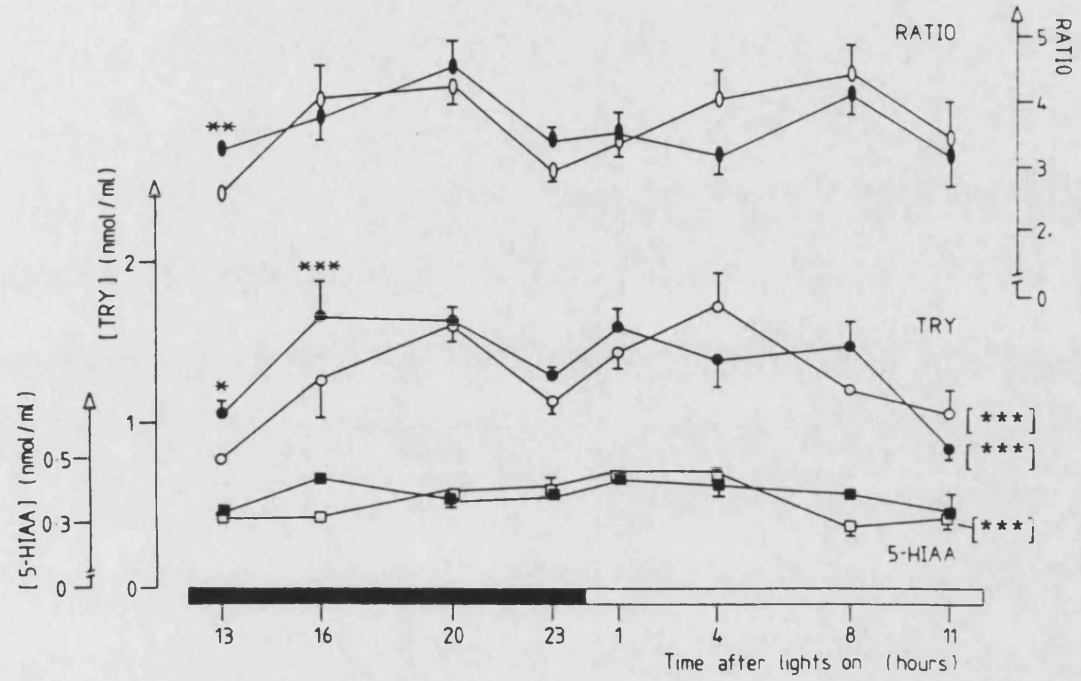


Figure 7.8 Effects of 7 x ECS on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF sampled 48 h after last treatment. Open symbols: control animals. Closed symbols: ECS-treated animals. Black bar on time axis: dark phase. Mean ± s.e.m.; n= 3-7, at each time point; *: $p<0.05$, **: $p<0.02$, ***: $p<0.01$ (t-test), compared to appropriate control; [***]: $p<0.01$ ANOVA.

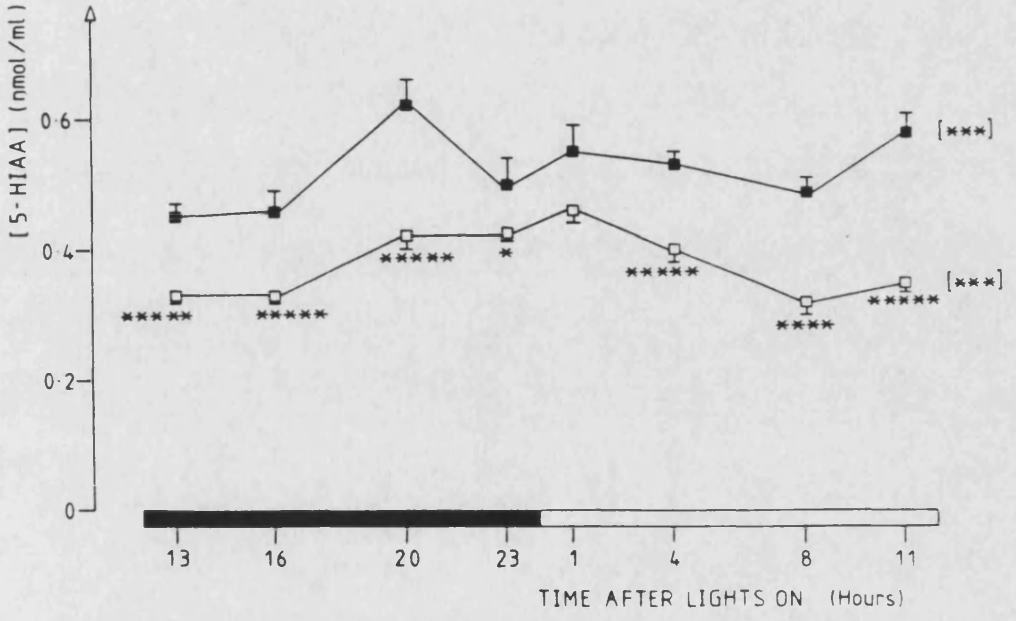


Figure 7.9 Effects of food deprivation on 5-HIAA concentration in rat CSF over 24 hours. Open squares: control animals n=11-16 : Closed squares: food deprived animals (n=3-6). Black bar on time axis: dark phase. Mean ± s.e.m.; *: p<0.05, ****, P<0.002, *****: p<0.001 (t-test), compared to appropriate control [***]: p<0.01, (ANOVA).

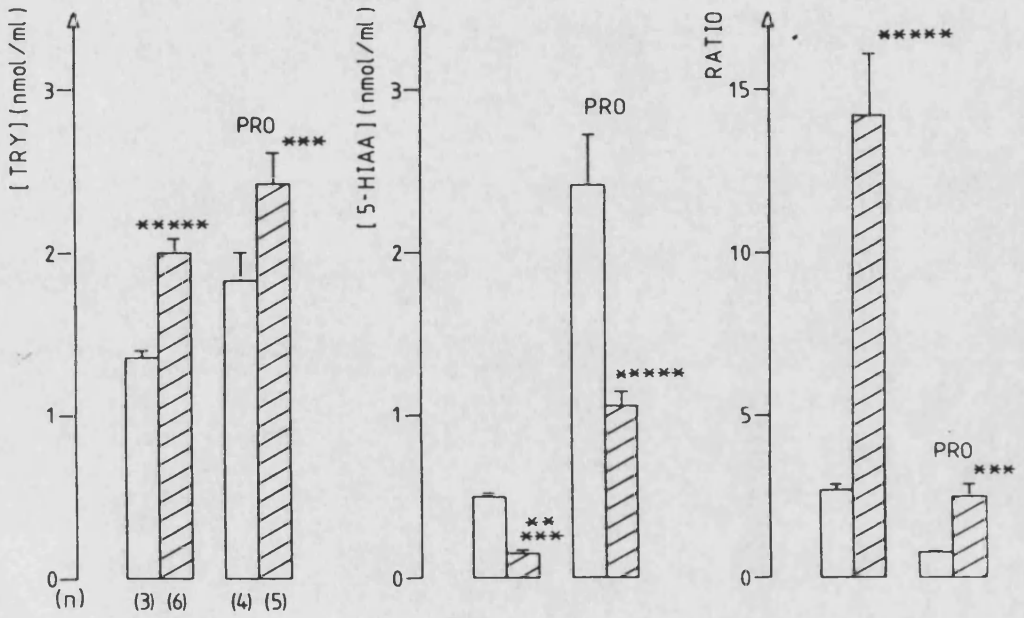


Figure 7.10 The effects of acute pCPA (150 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.; ***: $p<0.01$, *****: $p<0.001$ (t-test), compared to appropriate drug-free control.

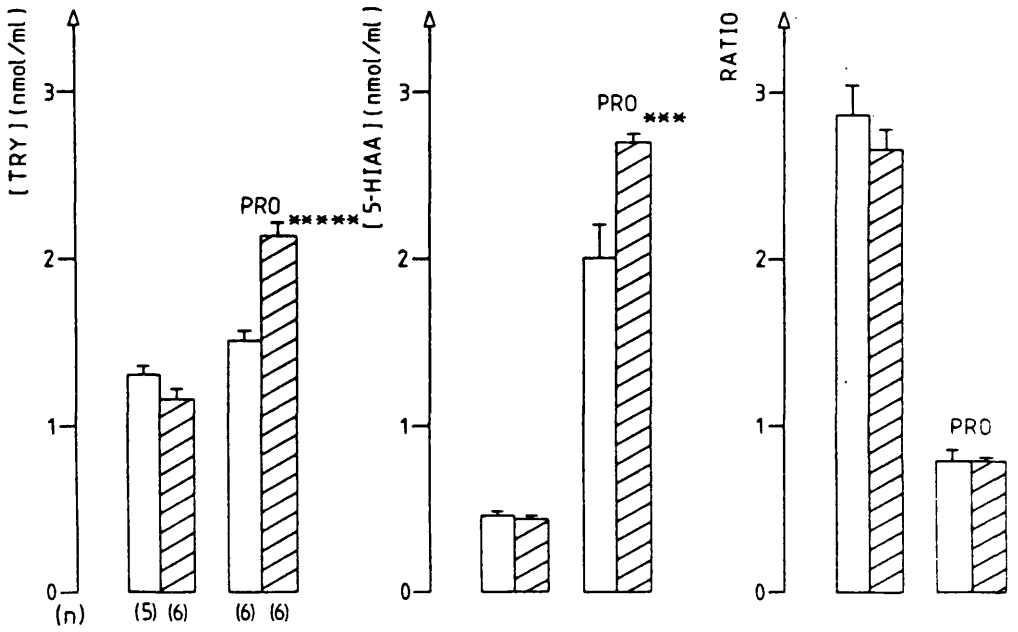


Figure 7.11 The effects of acute carbidopa (25 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean $\pm$ s.e.m.; ***: $p<0.01$, *****: $p<0.001$ (t-test), compared to appropriate drug free control.

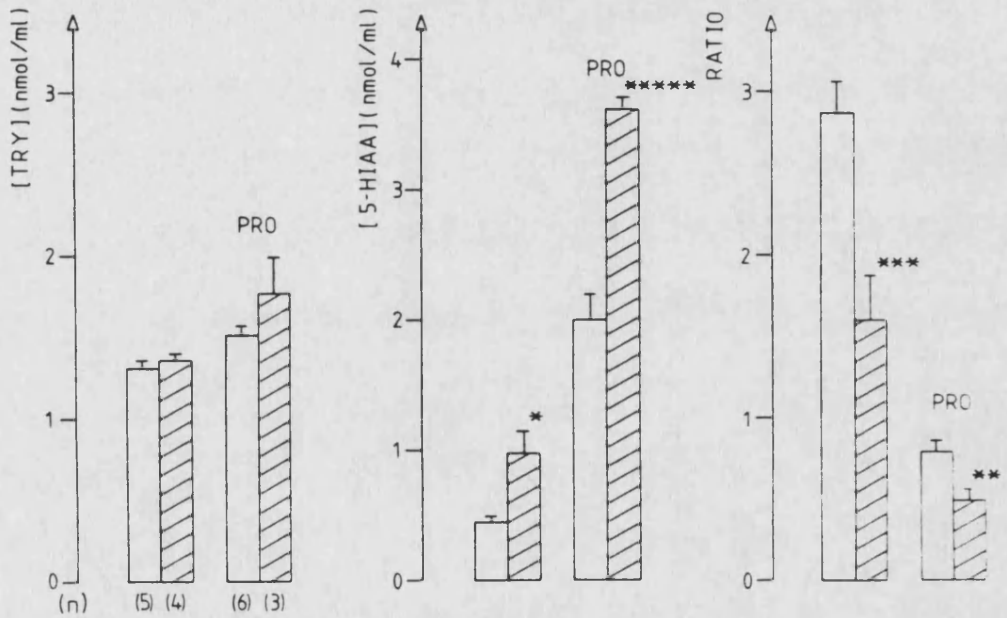


Figure 7.12 The effects of acute reserpine (5 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.; *: $p<0.05$, **: $p<0.02$, ***: $p<0.01$, *****: $p<0.001$ (t-test), compared to appropriate drug-free control.

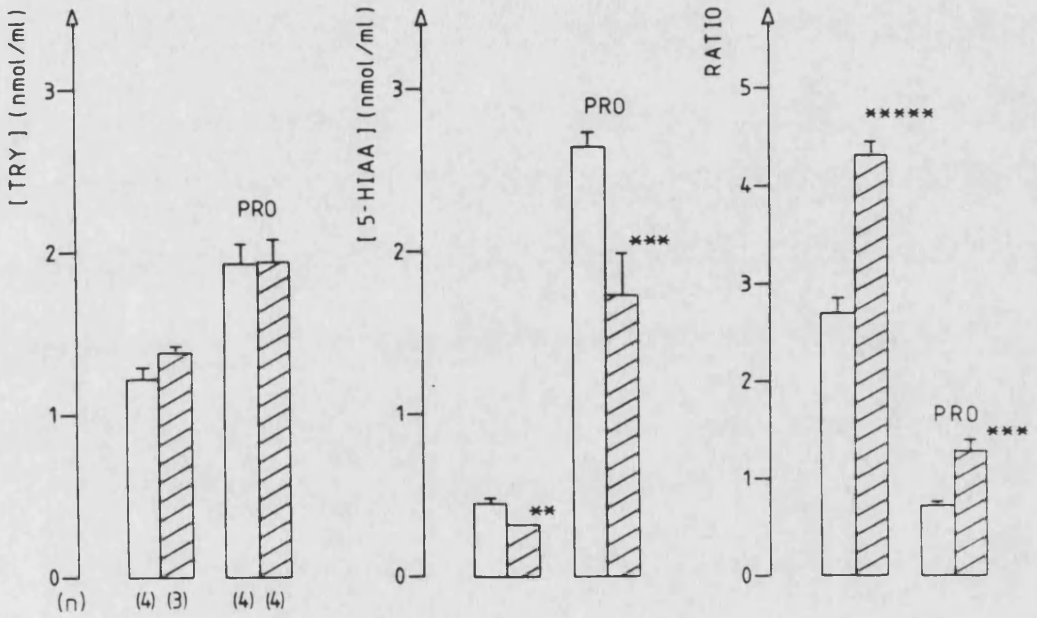


Figure 7.13 The effects of acute tranylcypromine (5 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m., **: $p<0.02$, ***: $p<0.01$, *****: $p<0.01$ (t-test), compared to appropriate drug free control.

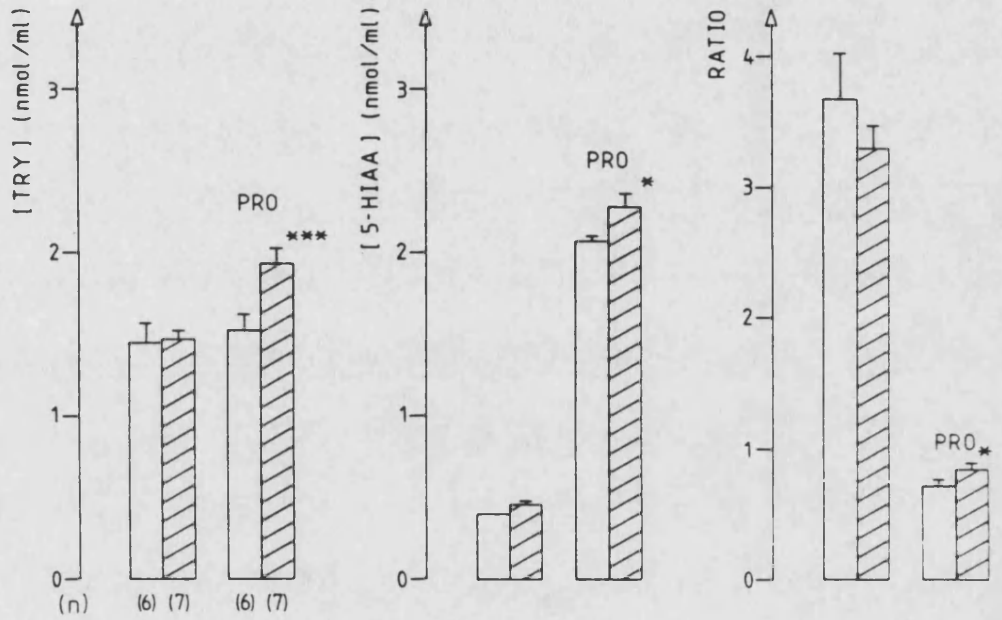


Figure 7.14 The effects of acute imipramine (20 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentrations and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.; *: $p<0.05$, ***: $p<0.01$ (t-test) compared to appropriate drug-free control.

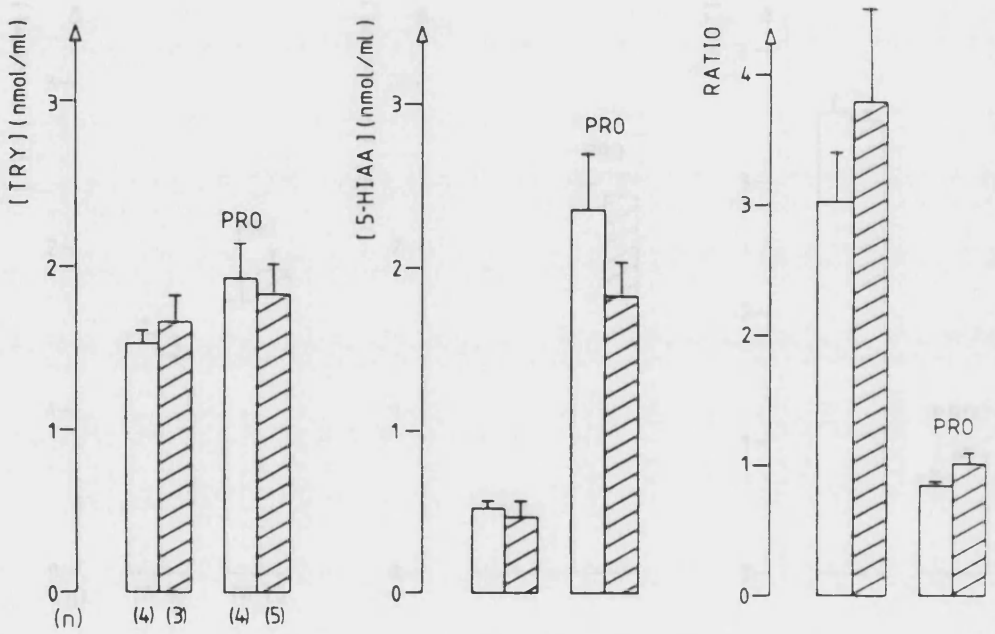


Figure 7.15 The effects of acute paroxetine (10 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.

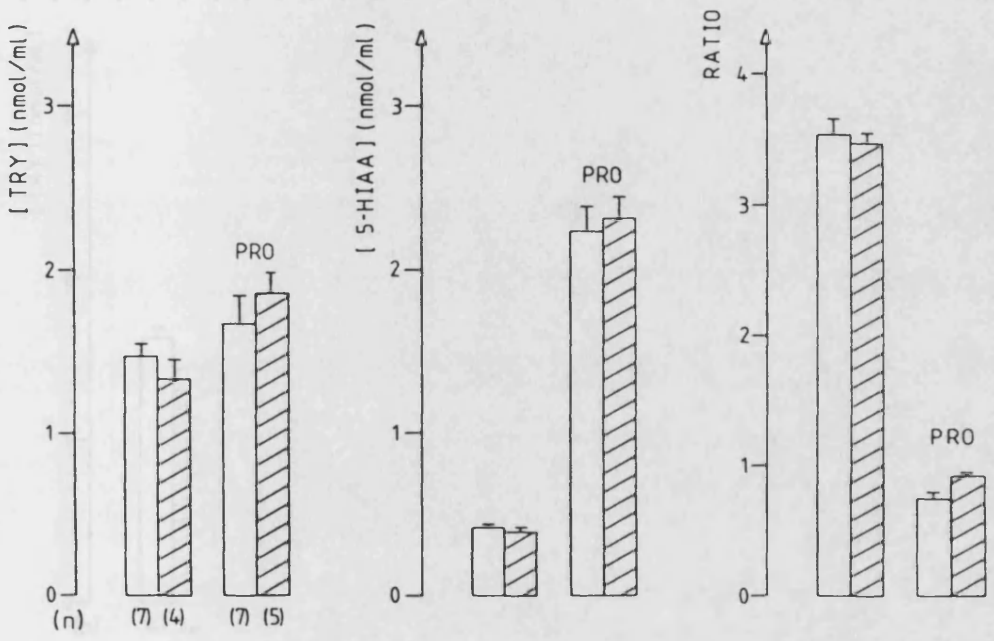


Figure 7.16 The effects of acute nomifensine (5 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.

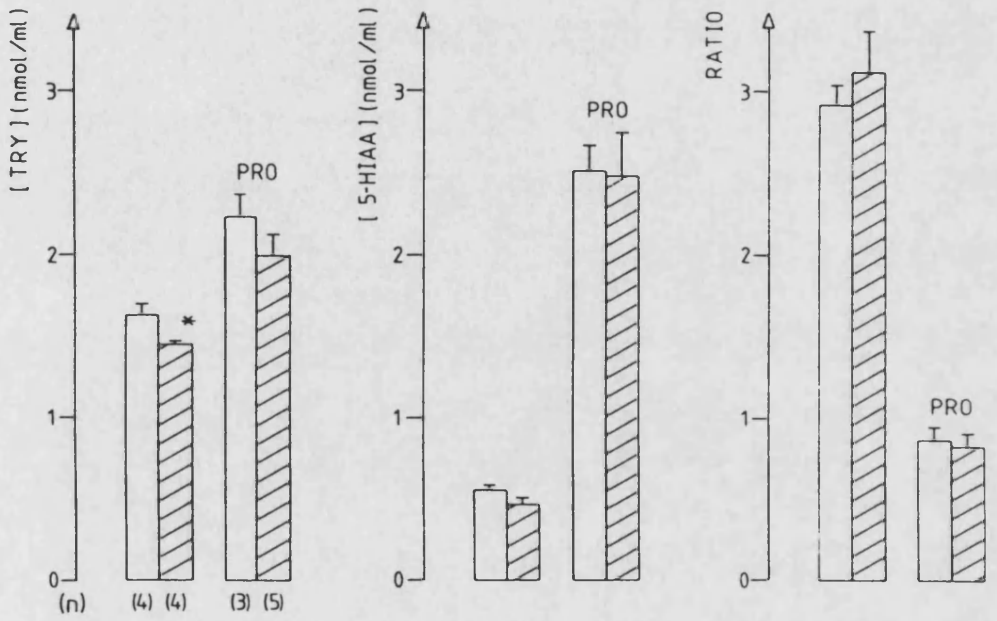


Figure 7.17 The effects of acute mianserin (10 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.; *: $p<0.05$ (t-test), compared to appropriate drug-free control.

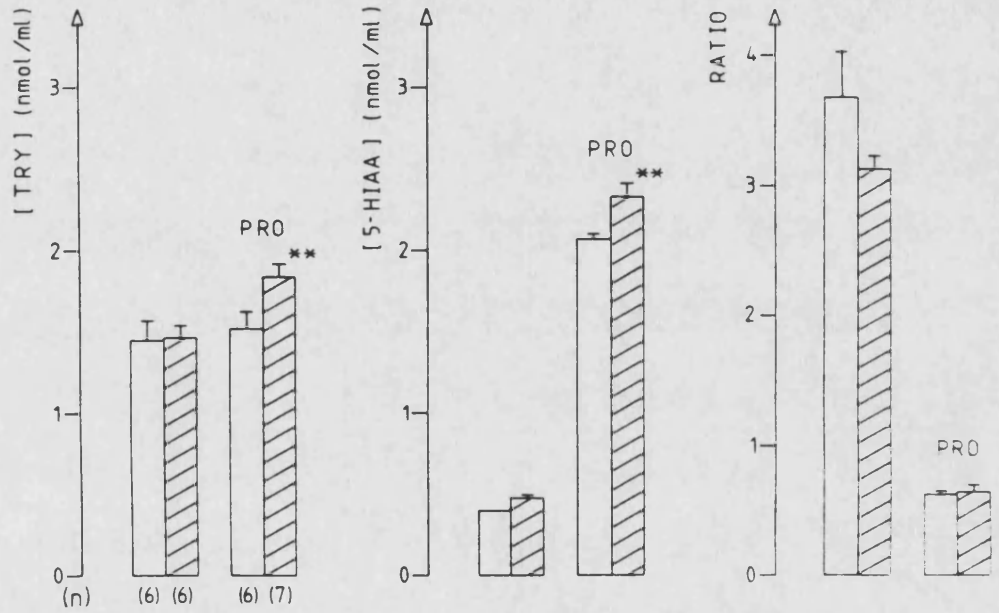


Figure 7.18. The effects of acute trifluperidol (10 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.; **: $p<0.02$ (t-test), compared to appropriate drug-free control.

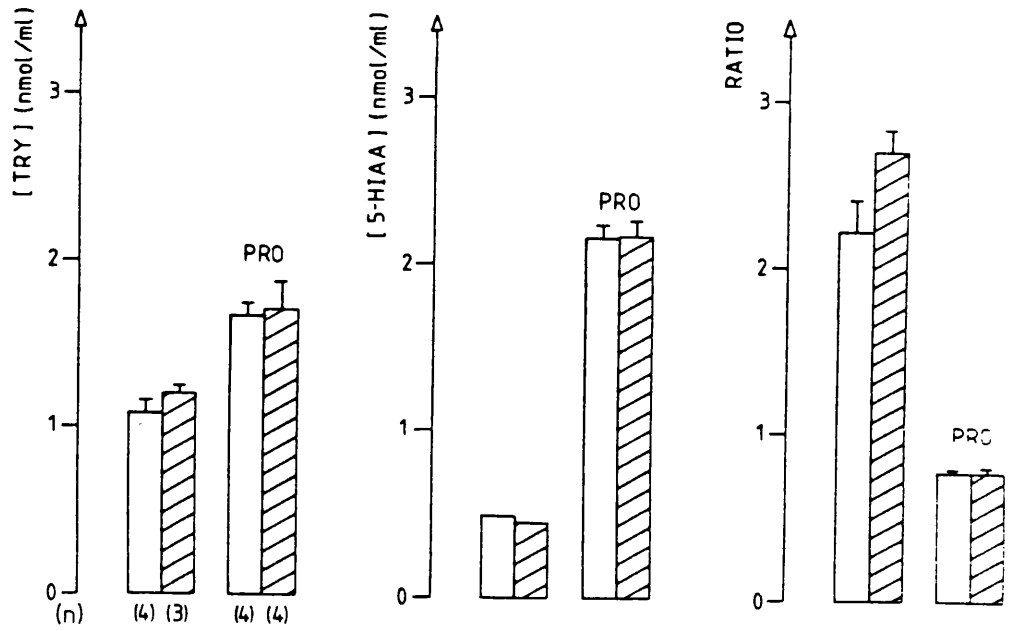


Figure 7.19 The effects of acute lithium (5mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentration, and their ratio, in rat CSF. Open columns: control animals. Hatched columns: experimental animals. Probenecid (PRO) administered 1 h before sampling. Mean ± s.e.m.

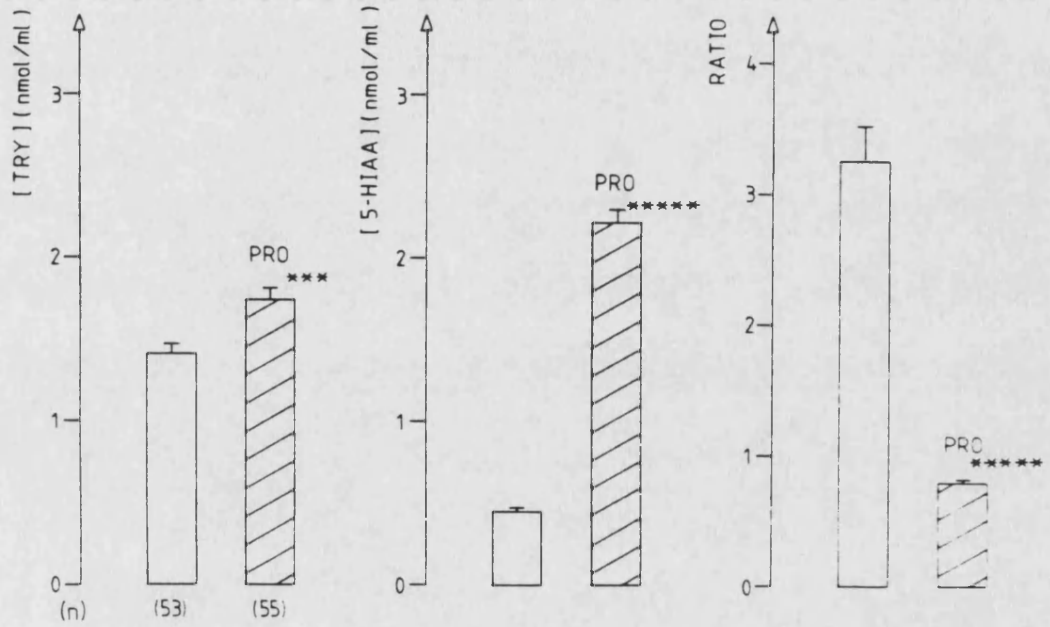


Figure 7.20 The effects of probenecid pretreatment (200 mg/kg, i.p.) on tryptophan (TRY) and 5-HIAA concentrations, and their ratio, in rat CSF. Open columns: vehicle-treated animals. Hatched columns: probenecid-treated animals. Mean ± s.e.m.; ***: $p<0.01$, *****: $p<0.001$ (t-test).

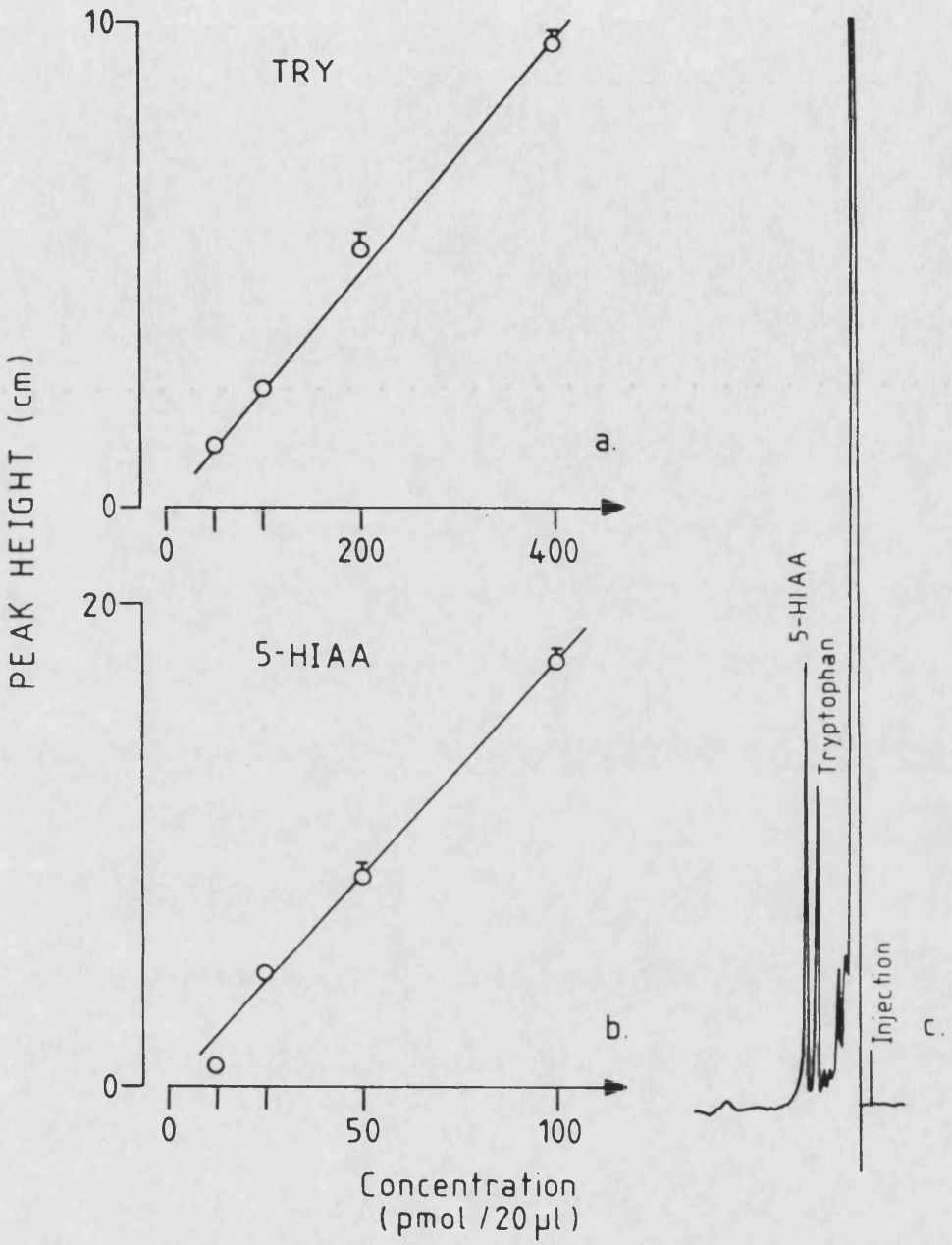


Figure 7.21 Relationship of peak height to concentration of (a) tryptophan and (b) 5-HIAA at +0.80V. Detector sensitivity: 10nA. Corr. Coef.: 0.99 for both substances. A typical chromatogram is depicted in (c).

two substances was proportionately decreased.

7.4. Discussion

(a) Daily rhythm of tryptophan and 5-HIAA concentrations

The first important finding from these experiments is that it is possible to identify a diurnal variation of tryptophan and 5-HIAA concentrations in rat CSF, without the use of probenecid. The rhythms are significant at the 99% confidence limit (ANOVA) and apparently in phase with each other.

Tryptophan concentration rose steadily during the dark phase, peaked 4 hours before lights on and then fell considerably before rising to a second maximum at 1 hour after lights on. Since tryptophan availability to the brain depends, among other factors, on diet and since food is consumed mainly during the dark phase, it is not surprising that brain tryptophan levels peak during the night in rats (Fernstrom, 1978). The source of CSF tryptophan is mainly the brain tissue and it could be expected that its levels would follow any increments observed in brain. Indeed, this is what the results indicate.

The peculiar pattern of the rhythm is not easily explained. It could be an artefact that would disappear if the best fit sinusoidal curve were applied. However, there is no physiological reason why any rhythm in an organism should fit a sinusoidal curve. Moreover, a very similar pattern was described for the diurnal variation of the head-twitch response in the mouse (Moser and Redfern, 1985), although the two peaks in the latter case surrounded the mid-light point (6 hours after lights on). The head-twitch response is, as we have

seen, thought to be mediated by 5-HT_2 receptors in the brain. Judging from the data of Kafka et al (1983) many neurotransmitter receptor population rhythms showed more than one peak during a 24-hour cycle, although, admittedly, in that study 5-HT_2 receptors were not examined.

The concentration of 5-HIAA also varied during the observation period; the concentration rose in phase with that of tryptophan, reaching a peak 1 hour after lights on. This, too, can be supported by the evidence that extracellular levels of 5-HIAA measured by in vivo voltametry were elevated during the dark phase (Cespuglio, Faradji, Crespi and Jouvet, 1982).

The observation that the two rhythms appear to be in phase may be coincidental. High 5-HIAA concentrations indicate increased 5-HT breakdown. If that presupposed greater synthesis rate, tryptophan levels could well be expected to be lower during the dark phase if utilization for 5-HT synthesis outstripped tryptophan availability or even matched the increased concentrations of tryptophan in brain.

While the present results seem to be fairly clear, other reports contradict them. In a similar study, Hutson et al (1984a) failed to find any variation of tryptophan or 5-HIAA concentrations in conscious animals but reported a variation in 5-HT turnover, calculated after probenecid pretreatment. In another two reports (Hutson, Sarna, Kantamaneni and Curzon, 1985; Sarna, Hutson, Tricklebank and Curzon, 1983), 5-HIAA was continuously measured for 4 to 5 hours in CSF but no significant variation with time was reported.

It can be argued that the results of Hutson et al (1984a) were based on only 4 time points whereas the results described here have

emerged from 8 time points. In the other two reports, sampling of CSF may have taken place during a period that 5-HIAA levels are, by chance, steady. Also, Hutson et al (1984a) have used conscious animals, although lack of anaesthesia would be expected to enhance, if anything, the appearance of a rhythm.

These are, of course, points of argument on methodological considerations. The crucial question is whether there is a physiological explanation for the daily variation of tryptophan and 5-HIAA. We saw that 5-HIAA concentrations most probably reflect 5-HT release, which is higher during the dark phase (Cespuglio et al, 1982). Regardless of whether enhanced release entails increased activity at 5-HT receptors, it certainly should result in higher 5-HIAA levels in brain. If the latter are not to lead to elevated 5-HIAA levels in CSF, one must assume either that there is a limitation to the amount of 5-HIAA that crosses from brain to CSF, the surplus being removed by the blood stream; or that there is a mechanism which rapidly eliminates 5-HIAA from CSF, maintaining it always at a constant level.

The first hypothesis is not consonant with the evidence that brain and CSF come into rapid equilibrium and that CSF is the major sink for brain 5-HIAA. The second one seems more plausible and would be reinforced by the fact that probenecid, which blocks the transport mechanism of 5-HIAA, induces an elevation in 5-HIAA concentration and could possibly reveal a diurnal variation.

On the other hand, if we accepted the argument that the transport mechanism is regulating the amount of 5-HIAA in CSF, it would be impossible to detect any changes caused by pathological

factors or pharmacological intervention; such changes are, however, detectable and of considerable clinical value. Moreover, the demonstration here, that food deprivation not only increases the concentration of 5-HIAA at all time points but also preserves the diurnal variation argues, convincingly, that CSF can be used to display the existence of a daily rhythm in 5-HIAA concentration, which, in all probability, reflects a rhythm in 5-HT release.

Finally, the significance of ratio of the two substances should be approached with caution. At first glance it would seem that there is a balance between tryptophan and 5-HIAA concentrations at all times. When tryptophan levels in blood were elevated, as for example after feeding during the dark period, brain levels were also elevated and CSF reflected that increase, as well as the concomitant increase in 5-HIAA. However, the two variables, the concentrations of tryptophan and 5-HIAA, may be independent. Tryptophan levels in CSF were elevated during the second half of the light phase and the first half of the dark phase, after food deprivation, but were normal during the remaining period. This is in agreement with reports of elevated brain tryptophan concentration following food deprivation (Chaouloff, Elghozi, Guezennec and de Laude, 1985; Curzon, 1981; Fernstrom, 1978). The fact that 5-HIAA levels were elevated at all times during the experiment and the ratio of tryptophan to 5-HIAA was within the normal range (data not shown) indicates that regulation of 5-HIAA concentration is essentially coupled to 5-HT release and not tryptophan availability.

(b) Effects of ECS

From the three series of experiments involving the

administration of acute or repeated ECS and sampling of CSF at various intervals after treatment, there is no evidence that ECS affects the metabolism of 5-HT. Since neither tryptophan nor 5-HIAA concentration was altered by ECS, it is assumed that tryptophan availability in brain, 5-HT synthesis and 5-HT release were not affected, to the extent that effects on these variables can be expressed through analysis of CSF.

It appears that there was no particular time in the 24-hour cycle that was more vulnerable to treatment, since at all time points, with rare exceptions, the levels of tryptophan and 5-HIAA were similar between experimental and control animals. Equally, it can be claimed that there are neither acute nor delayed effects of 1 x ECS since there were no changes observed when the ECS-sampling interval was 5 minutes, 3 hours or 24 hours.

In addition, none of the experimental protocols precipitated a temporally-uniform change in tryptophan or 5-HIAA levels, i.e. a change observed at all time points and pointing in the same direction.

As regards diurnal variation, again no effect can be attributed to ECS. The loss of rhythm for both tryptophan and 5-HIAA concentrations when CSF was sampled 24 hours after 1 x ECS was also found in control animals. It is difficult to imagine that halothane has such a detrimental effect, but one that is only detectable at 24 hours after 1 x ECS. The absence of a plausible explanation is reluctantly admitted and probably reflects a sampling artefact.

From a different point of view, however, a diurnal variation for 5-HIAA concentration was observed in the controls in all cases, but

was abolished at 24 hours after 1 x ECS and 48 h after 7 x ECS. Under these conditions, it may be assumed that ECS tended to affect the variation of 5-HIAA concentration. This assumption, however, would not provide an explanation for the loss of rhythm for tryptophan in both experimental and control groups, at 24 h after 1 x ECS. Clearly more experiments with larger group numbers are required to tackle the problem. Loss of the diurnal variation would inevitably involve steady concentrations over time, which would increase the possibility that experimental values differ significantly from control values. No such evidence or even trend was identified and it is, therefore, concluded that ECS does not affect the 5-HT system in a way that such an outcome is detectable by measuring tryptophan and 5-HIAA concentrations in CSF.

Two more possible targets of ECS to be discussed are monoamine oxidase and the blood-brain permeability.

A large number of ECS treatments is known to cause a persistent increase in MAO activity (Essman, 1978b; Fink, 1979; Pryor, 1974). This, in effect, should induce an elevation in 5-HIAA levels. Since no such increase was noticed it may be assumed that repeated ECS under the present schedule (7 x ECS within 13 days) was not sufficient to cause an increase in MAO activity.

Finally, there is evidence that cerebral blood flow and cerebrovascular permeability increase within minutes after ECS, possibly persisting up to 48 hours after treatment (Fink, 1979). Permeability to cocaine, which was increased after ECT, has been likened to the permeability characteristics of noradrenaline and 5-HT (Ottosson, 1974). From these observations changes in 5-HIAA levels in CSF would not be surprising. The lack of evidence in support of

this view may be better linked with the data of Webb, O'Donnell, Draper and Phillips (1984). These authors reported that there was no increase in serum brain-type creatinine phosphokinase, an enzyme found in very low concentration in serum, up to 6 hours after ECS, indicating that blood-brain permeability was unaltered.

(c) Acute drug administration

This series of pilot experiments was undertaken mainly to investigate the extent to which changes induced by agents with a well-established biochemical action would be manifested in CSF. From the results, it can be seen that drugs whose targets are located intraneuronally produced the expected effects. Thus, pCPA and tranylcypromine caused a reduction and reserpine an elevation of 5-HIAA levels. The ratio of the two substances reflected convincingly these changes.

On the other hand, administration of imipramine, paroxetine, nomifensine and mianserin had no effect on tryptophan and 5-HIAA concentration or their ratio, with the exception of a decrease in tryptophan concentration following mianserin. The lack of effect, apparently unrelated to the selectivity, or lack of it, of these drugs for 5-HT uptake inhibition was unexpected. The degree to which imipramine and paroxetine cause 5-HT accumulation resulting in elevation of 5-HIAA concentration, probably increases with number of doses, whereas acute administration may not be sufficient to elicit a change that is detectable in CSF.

No effect was observed after an acute dose of trifluperidol, a neuroleptic butyrophenone with antidopaminergic action. Finally, lithium also failed to produce any changes. Chronic treatment with

lithium enhances tryptophan uptake by the brain and inhibits 5-HT uptake by the neurons (Knapp, 1983). Neither of these effects could be connected with a single dose of lithium, which was totally without effect.

(d) The use of probenecid

The results in figs. 7.1-4 clearly indicated that probenecid pretreatment caused a significant increase in tryptophan concentration. This conclusion was strengthened by the collective results from acute drug administration, which also showed that probenecid administration effectively increased tryptophan concentration in CSF (Fig. 7.20).

Probenecid is thought to displace tryptophan from albumin in plasma and thus increase its availability to, and uptake by, the brain. Even if the increase leads to only minor changes in 5-HT synthesis and, consequently, 5-HIAA levels, one could argue that the method should not be used when tryptophan or 5-HIAA concentation is determined. Strictly speaking neither concentration can be relied on unless the degree of probenecid-induced alteration in synthesis and the extent of change induced by the treatment under investigation are known beforehand. The present results, therefore, challenge the view that sampling of CSF within 1 hour after administration of 200 mg/kg probenecid is unlikely to upset the correct estimation of tryptophan and 5-HIAA levels and 5-HT turnover (Curzon, Hutson, Kantamaneni, Sahakian and Sarna, 1985).

Along with these arguments against the use of probenecid, the results provide three sets of data that might question this

conviction. In the experiments with carbidopa, imipramine and trifluperidol, there was an increase in both tryptophan and 5-HIAA concentration, in animals pre-treated with probenecid, followed by one of these drugs, compared to control rats treated with probenecid alone. The degree of 5-HIAA concentration increase in the probenecid-treated controls shows that probenecid was sufficiently absorbed and that the answer to the puzzle does not lie with the kinetics of probenecid. One possible explanation is an interaction of probenecid with each one of these drugs, although there is no obvious common link between carbidopa, imipramine and trifluperidol that can explain the findings. This discrepancy might be resolved and more valuable data would be collected, if these experiments could be repeated over a dose range and also compared to the effects of chronic administration.

(e) Methodology

Although the method of CSF sampling, as described here, appears to have yielded dependable results, the need and value of systematic sampling from the same, conscious animals throughout a 24-hour cycle and for many days cannot be overemphasized. In order to evaluate the validity of the data obtained from the present method, two more techniques have been used.

The first one has been described by Sarna et al (1983) and is one of the simplest methods described in the literature for sampling CSF from conscious animals through permanent catheters, implanted in the cisterna magna. The second technique is basically a variation of the one used here and consists of leading a cannula through a tiny hole in the skin, through the musculature and into the cisterna magna, under anaesthesia. After sampling, the animal is allowed to

recover and can be used repeatedly, but with long pauses between successive samplings.

The major problems encountered with the first method were a low initial success rate and, more importantly, a high percentage of blocking or dislocation of the catheters. Even if the method could be improved and used successfully, it could not be coupled to probenecid pretreatment, since the latter is associated with toxic effects, following repeated administration (Hutson et al, 1984b).

The second method is quite successful but also presents a problem, in that it requires that an analgesic and antibiotic be administered, thus introducing possible unwanted drug interactions.

Nevertheless, both methods have been used on a small scale and the values obtained for tryptophan and 5-HIAA (not shown here) were well within the range of values obtained by the method employed throughout these studies.

Finally, halothane anaesthesia does not seem to have any effects on the variables measured. Determination of tryptophan and 5-HIAA in conscious animals and halothane-treated controls yielded identical results, and chronic exposure to halothane was devoid of effect.

(f) Final conclusions

Several conclusions can be drawn from this discussion. The main finding was that there is a diurnal variation in the concentration of tryptophan and 5-HIAA in rat CSF. Acute and chronic ECS failed to alter either the standing levels of these substances at any time point examined, or the characteristics of their variation with time. Probenecid introduces a bias in the estimation of tryptophan and

5-HIAA levels. Finally, preliminary results with a single dose of various drugs were characterized by inconsistency, necessitating further investigations, but did serve to indicate that measurable changes in CSF concentrations of tryptophan and 5-HIAA could be induced and estimated by the present method.

The design of these experiments affords the estimation of tryptophan and 5-HIAA concentrations and their temporal variation, from which certain valid deductions can be made on the turnover of 5-HT. The correlation of tryptophan with 5-HIAA, using their ratio in CSF, may be misleading and should, therefore, be avoided.

CHAPTER 8 THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN MICE AND RATS - EFFECTS OF ECS AND ANTIDEPRESSANT DRUGS

8. THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN MICE AND RATS - EFFECTS OF ECS AND ANTIDEPRESSANT DRUG

8.1. Aim

The presence of a circadian rhythm in locomotor activity in rodents is well established and has been shown to be susceptible to changes by antidepressant drugs (section 3.6.1). The possibility that ECS, too, could influence the locomotor activity rhythm in rats and mice (and in either entrained or freerunning condition), as part of its mode of action, has been investigated. A limited number of experiments involving administration of antidepressant drugs were also conducted in order to compare the responses to the two different treatments.

8.2. Materials and Methods

8.2.1. Animals

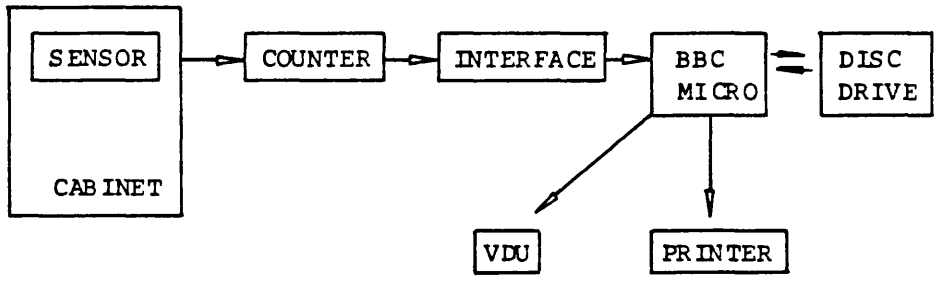
Male WISTAR rats and male CFLP mice were used in these experiments. The age of animals at the beginning of the experiment was usually 3 to 4 weeks (mice and rats) and their weight 15-25 g (mice) or 90-120 g (rats). All experiments were terminated before the animals reached 10-11 weeks of age.

8.2.2. Housing Conditions

All the experiments included in this chapter were performed in the environmental cabinets, as described in section 6.3., which accommodated the appropriate equipment for monitoring locomotor activity.

8.2.3. Monitoring of Locomotor Activity

Locomotor activity for mice and rats was measured by VARIMEX horizontal activity electromagnetic sensors (Columbus Instruments Ltd), placed in the cabinets, underneath the plastic animal cages. A signal from the sensors was picked up by an activity counter (Columbus Instruments Ltd) and stored on a computer disc. The complete network is presented schematically below:



Activity scores were aggregated every fifteen minutes, for 24 hours daily during each experiment. Details of the programme which enabled the storage and analysis of the data have been presented by Marshall, Sparks and Davies (1984). The data accumulated from these recordings is presented in three forms. First, it can be shown in the form of actograms (Fig. 8.1a) which display the daily activity distribution over 24 hours, double-plotted to facilitate visual inspection. Each bar in the actogram represents a sampling interval with a number of counts higher than the threshold used in the plotting of the actogram. Thus, it is not so much an absolute measure as an aid to identify the pattern of a rhythm. Selected actograms are presented whenever they are required to illustrate a point. A second method is the daily activity plot which offers an aggregate of the counts on each day, in the shape of a histogram (Fig. 8.1b). Instead of including all the original plots obtained from the computer, the average of

daily activity at different periods during an experiment, expressed as mean ± SEM, was used and compared by Student's t-test.

A third way of analysing the data was by subjecting the data to a time series analysis which enabled the identification of the frequencies of the rhythms. Since the method does not provide a threshold that can differentiate between real rhythms and noise, an arbitrary threshold was used as follows: all peaks with a power less than 20% the power of the main peak were discarded. Peaks with values betwen 20% and 50% are considered secondary and quoted in parenthesis, and peaks that exceed 50% are rated main frequency peaks (Fig. 8.1c). To save space, the spectral analysis result were presented in a tabular form, as exemplified in Figure 8.1d.

8.2.4. ECS and Drug Treatment

ECS was administered every second day, including weekends, to a total of 7 treatments (7 x ECS), as described before. Appropriate controls (sham ECS) were used in all cases.

Drugs were administered orally in the drinking water. Water consumption was usually measured daily, since it did not involve disturbance of the animals; fresh drug solutions were also daily provided.

8.2.5. Experimental Schedule

As a rule, whenever a cycle other than the normal L:D cycle was employed, a period exceeding one week was allowed before the initiation of treatment. The time of ECS administration is discussed in the individual experiments. After the end of treatment, a few days

were allowed (post-treatment period), during which activity was still monitored, before the experiment was terminated. Although suitable controls were always used, the animals served mainly as their own control.

8.3. Results

8.3.1. Repeated ECS During the Inactive Phase of a Normal L:D Cycle

(a) Rats

Repeated ECS was administered 2 hours or $9^1/_2$ hours after lights on. During the post-treatment period, the mean daily activity of the experimental groups was unaltered compared to that of the treatment period. One of the control groups showed a considerable increase in mean daily activity during the post-treatment period (Table 8.1). Spectral analysis showed that the activity of all groups had a dominant component at 24 h and a secondary component with a period of 8 h. Also, there was a tendency for both experimental and control groups to move from the basic period of 24 h towards 23 h (23.50 ± 0.50h), although visual inspection of the actograms did not support such a finding.

(b) Mice

Essentially the same picture emerged when mice were used instead of rats and treated at 7 or 12 hours after lights on (Fig. 8.2). There was no change in mean locomotor activity in the two control groups or the experimental group receiving ECS at 7 hours after lights on (Table 8.2). A decrease in activity was observed post-treatment, when the animals were subjected to repeated ECS at 12 hours after lights on. However, since the post-treatment period was

fairly short, the initial decrease in activity might disappear if the observation time after treatment increased to a week or more. From the spectral analysis it was seen that all groups behaved essentially like the rats: the predominant period was 23.50±0.25 hours. Since visual inspection of the actograms do not show any deviation from 24 hours, it may be assumed that a fluctuation of ±0.50 h is intrinsic to the recording method and/or the time series analysis used.

8.3.2. Repeated ECS During the Active Phase of a Normal L:D Cycle

(a) Rats

Repeated ECS was administered at 17 or 21 h after lights on. Compared to mean activity during normal L:D, the entraining phase to reversed L:D led to a moderate increase in activity (Table 8.3). Compared to the pretreatment period, the mean activity of both control and experimental animals increased significantly during the treatment period, an effect sustained for a week following termination of procedures. Thus no effect could be attributed to ECS (Fig. 8.3). The prevailing periods were in the band of 24-25 hours, higher during the entrainment period. A secondary period of 8.00 h was frequently observed.

(b) Mice

When the same experiment was performed on mice treated with ECS 16 after lights on, it was found that both control and experimental animals showed a decrease in mean daily activity during treatment period compared to a 5-day pretreatment time (Table 8.4). To the

extent that the decrease is not an artefact due to the small number of pretreatment days, it was sustained during the post-treatment period, reaching statistical significance in the experimental group. However, again no effect attributable to ECS could be detected. The entrained rhythm had a stable period of 24.00 to 24.25 h and the experimental group also displayed a harmonic of 8h during the majority of the observation period.

8.3.3. Repeated ECS on Animals Freerunning in Constant Dark

(a) Rats

In a preliminary experiment with groups of three rats it was impossible to obtain a freerunning rhythm under conditions of constant dark. All groups maintained a 24-hour rhythm with almost no deviation. Since it was suspected that cohabitation might obstruct the development of a freerunning rhythm, one rat per cabinet was kept under identical conditions. The modification yielded a much clearer pattern (fig. 8.4) and a possible explanation for this event is offered in the discussion.

The transition from a normal L:D cycle to constant darkness appears to have caused a large increase in locomotor activity, in all cases (Tables 8.5a,b).

The animals were treated with ECS either at $2^1/_2$ (Fig. 8.4 and Table 8.5a) or $9^1/_2$ hours (Table 8.5b) after the original "lights-on" time. Since the shift of the rhythm was minimal (as will be seen in the next paragraph), treatment in the first case corresponded with the end of the active phase and in the second with the middle of the inactive phase. The rat treated with ECS at $2^1/_2$ h after lights-on showed a significant increase in locomotor activity during the

inactive phase of the treatment period (fig. 8.4), whereas the rat treated at $9^1/_2$ hours and the two separate control animals did not quantitatively change their activity. It is possible that when rats were disturbed (for treatment) during their inactive phase, anticipatory activity was generated, persisting for a week after treatment.

Visual inspection of all actograms indicated that the freerunning rhythm may have been entrained by halothane administration during the active phase. Spectral analysis revealed that, during the initial L:D phase the prevailing period is around 23 h, increasing to 24 h during freerunning conditions.

It cannot be conclusively said that ECS increased locomotor activity or altered the prevailing period. What can be speculated, based on the spectral analysis is that the maintenance of single rats in conditions of continuous darkness led to the appearance of secondary periods (4-8h) which often dominated the spectrum. It is impossible to discern from the available data whether constant dark or social isolation is the main reason for this effect.

(b) Mice

The response of mice to conditions of total darkness was remarkably different from that of rats. First, repeated ECS significantly decreased locomotor activity during the same period that control animals showed an increase in activity (Table 8.6). However, when the treatment was over, activity in both groups returned to the same levels as before the treatment period, indicating that, whilst ECS might have had a genuine inhibitory effect, handling of

the control group or, possibly, halothane had a positive influence on activity.

The second difference was that mice tended to freerun with a period smaller than 24 hours (fig 8.5). Visual inspection would leave little doubt that both rhythms have a frequency below 24 h and spectral analysis supported this conclusion.

8.3.4. Repeated ECS on animals freerunning in constant light

(a) Rats

The transition from a normal entrained rhythm to continuous light produced an increase of up to 100% in locomotor activity in all groups of animals involved (Tables 8.7a,b).

Repeated ECS was administered at a time point corresponding to $3^1/_2$ hours after lights on at the original L:D cycle. That meant in effect that the first treatment took place at roughly the first third of the active phase and subsequent treatments were spaced at increasing intervals before the beginning of the active phase, as the latter drifted to the right (Fig. 8.6). In the other two groups, treatment initiation coincided with the end of the active phase, ($9^1/_2$ after original lights-on time), and so the last treatment took place at the beginning of the active phase.

In the first case, with the time of treatment crossing the inactive phase ("advanced" treatment) there is an indication of anticipatory activity in both control and experimental groups (fig 8.6). This activity is inevitably masked when treatment takes place during the active phase, ("delayed" treatment).

The repeated ECS treatment failed to produce any further increases in activity. Also, one control group (Table 8.7a) was

characterised by a highly significant increase. The "advanced" treatment (coinciding with the inactive phase) caused a slowing of the freerunning rhythm. The control group also displayed a slowing down in frequencies though not as large as in the ECS group. After one week in constant light, secondary peaks began to emerge in the period spectrum with values mainly between 3 and 7 hours. In the case of the control group, peaks of 5.25 and 6.00 hours, along with the main 25.00 h peak, dominated the spectrum during the 4th and 5th week of the L:L period. Although frequencies at the same band emerged in the experimental group they did not reach the 50% mark (Table 8.7a).

During the "delayed" treatment, repeated ECS again appeared to disrupt the freerunning period, but not to a significant degree. On the contrary, the corresponding control group was characterized by a complete breakdown to ultradian periods in the 4-6 hour band, with a prevalence of the 5.00 and 5.25 h peaks which are roughly harmonics of a basic period of 25.25 h (Table 8.7b).

Taken as a whole, ECS may have slightly slowed down the freerunning rhythms in rats, although it has not altered, quantitatively, locomotor activity. Since the two control groups presented a tendency to break down to ultradian components of activity, it is tempting to speculate that ECS played a proactive role in the experimental animals, preventing the disruption caused by the, admittedly not exceptionally prolonged, maintenance of rats under conditions of constant light.

(b) Mice

As was the case with rats, two time pointswere used in the freerunning cycle in mice. The first was at 5 h after lights on with respect to the original cycle, the first treatment taking place at the end of the active phase and the last coinciding with the beginning of the inactive ("advanced" treatment). The second time point corresponded to 12 h after lights on, treatment started at the beginning of the inactive phase and crossed the active phase, the last one taking place in the end of the shifted inactive phase (fig. 8.7).

The interval from the change into constant light to initiation of treatment was only 1 week and thus any comparisons between the pre-treatment and treatment periods may be invalid.

With the "advanced" treatment, the activity during the post-treatment period was greatly enhanced in both control and experimental groups, compared with levels during treatment. The rhythm of both groups in "advanced" treatment displayed a fairly steady period of about 25.00 h (Table 8.8a).

On the contrary, the period of the freerunning rhythm of the animals in the "delayed" treatment groups showed an increase and also, during the post-treatment period, a second peak, which approached the value of the first harmonic of the main period (26.00 h) was present in both groups (Table 8.8b).

Visual inspection of the actogram for the control group in fig. 8.7 indicates that a second burst of activity begins to appear after about day 16. It is possible that the peak with a period of about 13 h corresponds exactly to the period of this block of activity. The experimental group did not present an equally clear picture. In

all four groups, some kind of anticipatory activity seems to have developed.

In conclusion, repeated ECS on mice in constant light tended to decrease locomotor activity, which increased again after treatment. On the other hand, the frequency of the freerunning rhythm tended to decrease with the "delayed" treatment, an effect not specific to ECS, since the control group also showed the same effect (fig. 8.7).

8.3.5. Chronic Paroxetine and Mianserin - Effects on the Freerunning Rhythm in the Mouse

Two groups of mice were dosed for 11 days with either mianserin or paroxetine. Both drugs were given in the drinking water in concentrations designed to deliver about 10 mg/kg b.w./day. Water consumption was frequently monitored and an essential assumption was made that each animals consumed an equal amount of water daily.

Compared to control group, both paroxetine and mianserin induced a significant increase in locomotor activity, which was sustained during the washout period and more pronounced with paroxetine (Table 8.9).

Visual inspection of the actograms ensured that the control and the two experimental groups were freerunning normally during the experiment, although the control group showed signs of deviation from the standard picture. Spectral analysis indeed revealed two significant peaks at 13.00 h and 26.25 h during the period corresponding to oral dosing. The mianserin group freeran with a period between 26.75 and 26.00 hours, i.e. within the normal limits. The paroxetine group, however, freeran with the slow period of 28.00 h during

treatment, which decreased by 1 h during the post-treatment period.

Thus, it appears that paroxetine, but not mianserin, may slow the freerunning rhythm in mice under continuous light, whilst both drugs may cause a substantial and sustained increase in locomotor activity.

8.3.6. Chronic Tryptophan, Clomipramine and Imipramine - Effects on Freerunning Rhythm in the Mouse

Mice were given tryptophan, clomipramine or imipramine in their drinking water, for 15 days. The concentrations of the drug solutions were calculated to give 100, 50 and 50 mg/kg b.w./day of the three drugs respectively.

Unforeseen circumstances necessitated the use of two animals per group, and only one mouse in the tryptophan group. However, other experiments with the normal number of animals per group yielded the same results.

During the course of the experiment, the activity of the control group was increased, in agreement with the majority of the previous results (Table 8.10). Transition to the original L:D cycle induced a 3-fold increase in activity. The three drugs employed in this experiment produced very mixed results. Tryptophan did not significantly change locomotor activity, despite an apparent trend to increase it. Clomipramine produced a significant decrease and imipramine a significant increase (Table 8.10). These effects were sustained for all groups during a brief washout period under L:L. When the light cycle was returned to normal L:D 14:10 and drug solutions were removed, the 3 drug groups again behaved differently. The single mouse on tryptophan showed an almost 3-fold increase in

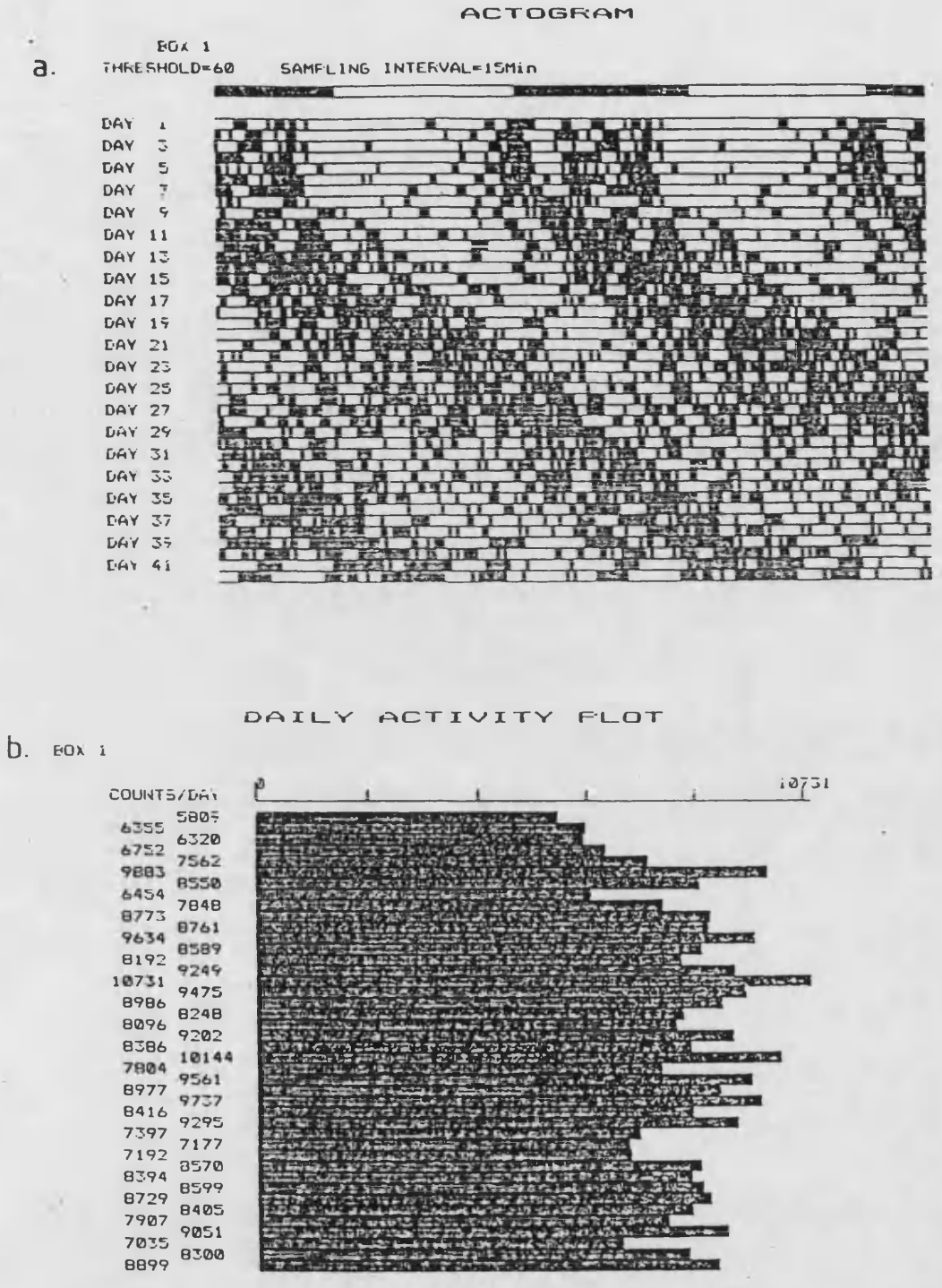
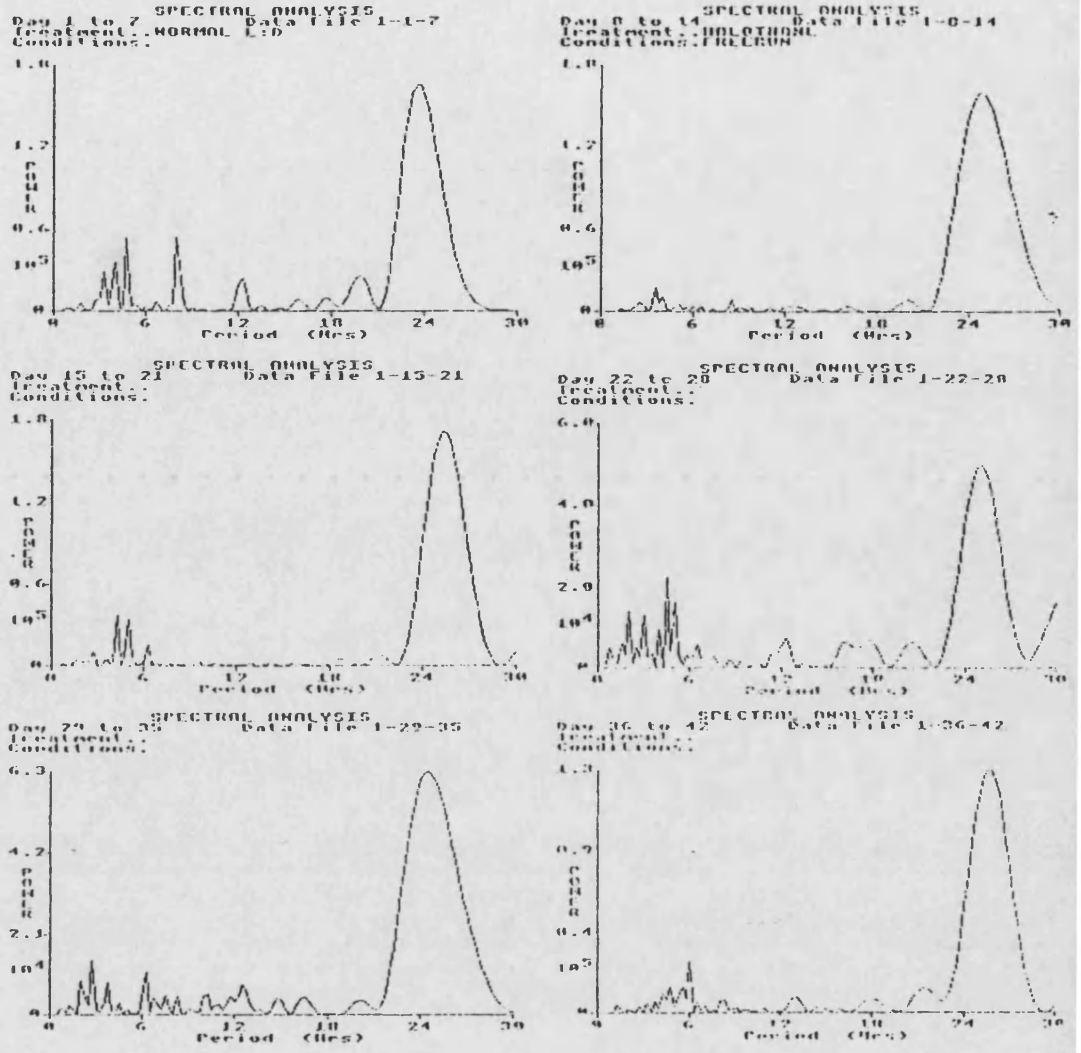


Figure 8.1 An example of data presentation for locomotor activity rhythms. (a) Locomotor activity rhythm (actogram) of rats freerunning in constant light, double-plotted. (b) Daily activity plot of the same animals. The horizontal light-dark bar should not be taken into account.

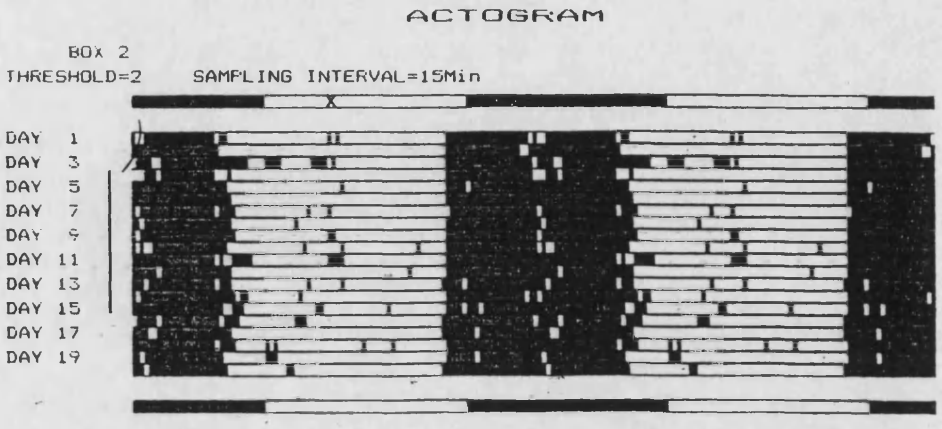
c.



d.

Days	Predominant Periods	
1-7	(4.00)(4.75)(8.00)	23.50
8-14		24.75
15-21	(4.25)(5.00)	25.25
22-28	(2.00)(3.00)(4.50)(5.00)	25.00
29-35	(2.75)	24.50
36-42	(6.00)	25.50

Figure 8.1 (c) Spectral analysis of the same data (as in Figure 8.1a and b). (d) Results from the spectral analysis in (c), presented in a tabular form. The absolute power values for the peaks of each plot were also obtained, from which the main and secondary peaks were identified.



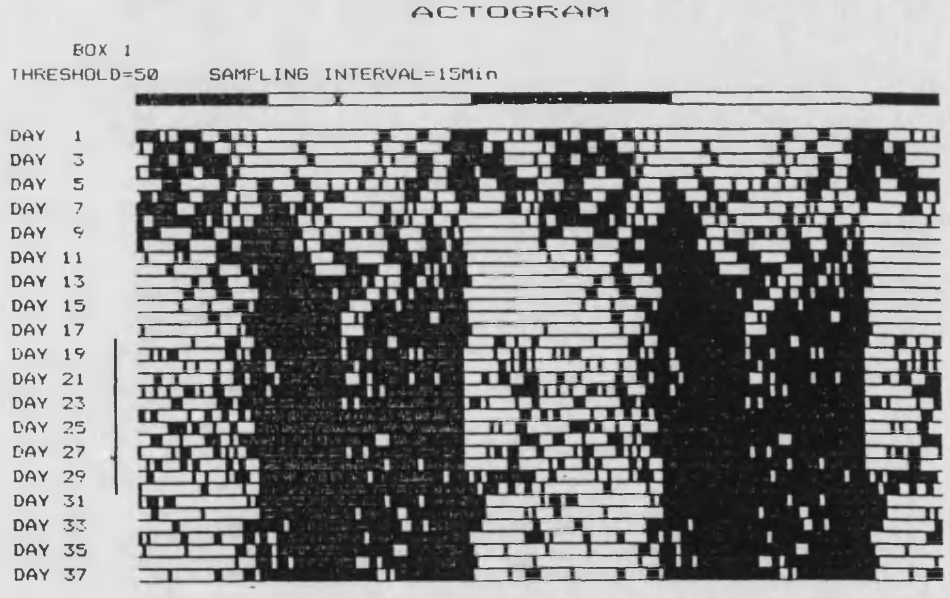
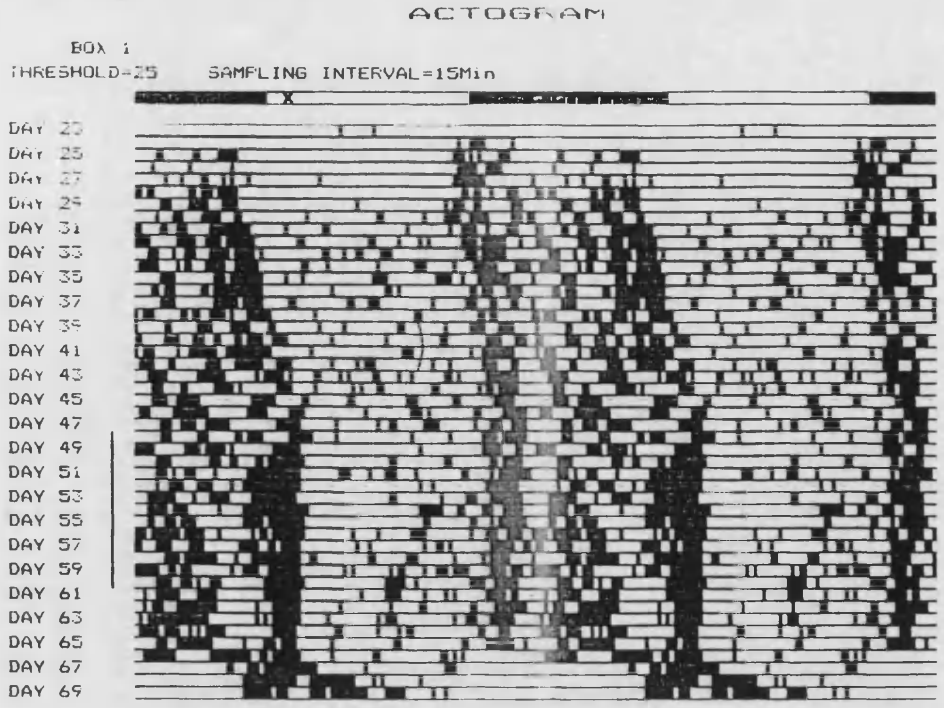


Figure 8.2 Locomotor activity rhythm of mice on a normal L:D 14:10 cycle. The animals were subjected to sham ECS between days 3 and 15, at 7 h after lights on (x).

Figure 8.3 Locomotor activity rhythm of rats entrained to a D:L 12:12 cycle. ECS was administered between days 18 and 30, at 17 h after lights on (x).



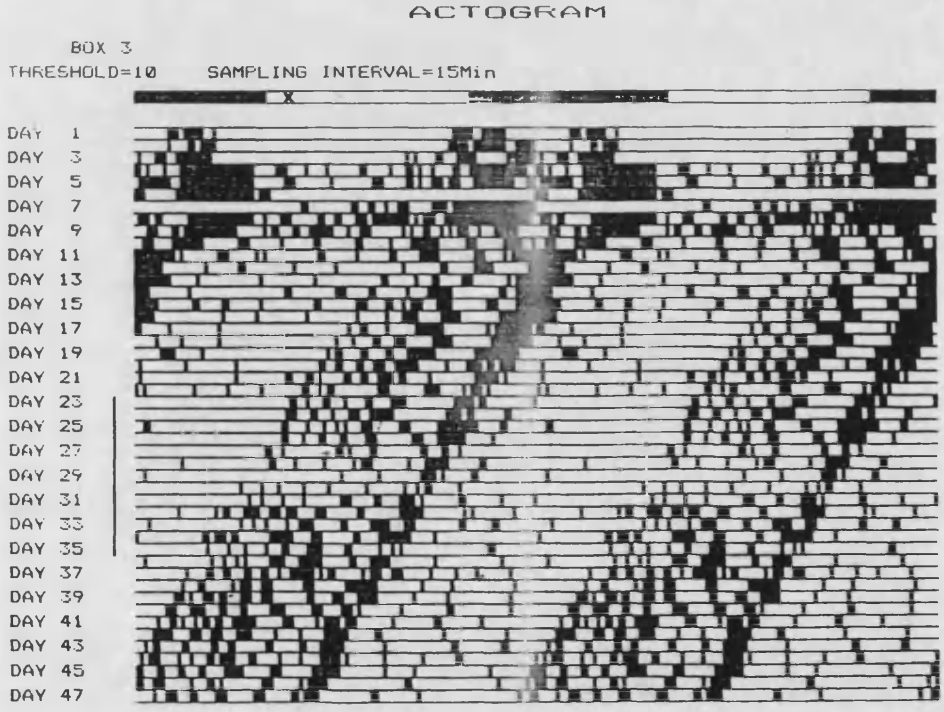


Figure 8.4 Locomotor activity rhythm in rats freerunning in constant dark since day 28 and subjected to sham ECS between days 48 and 60, at 2½ h after the original "lights on" time (x).

Figure 8.5 Locomotor activity rhythm in mice freerunning in constant dark since day 3 and subjected to ECS between days 23 and 35, at 4½ h after the original "lights on" time (x).

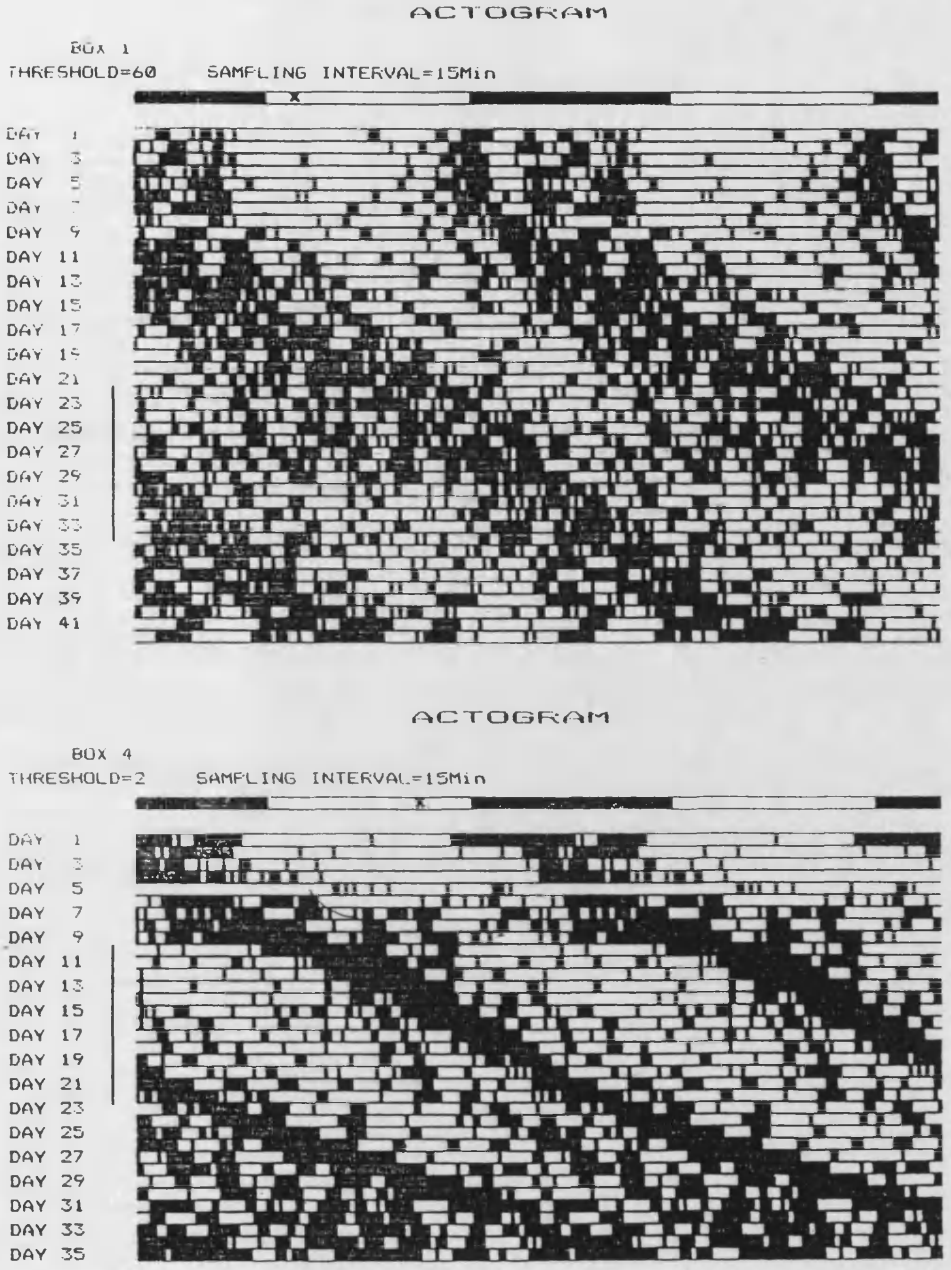


Figure 8.6 Locomotor activity rhythm in rats freerunning in constant light since day 7 and subjected to ECS between days 22 and 34, at 3½ h after the original "lights on" time (x).

Figure 8.7 Locomotor activity rhythm in mice freerunning in constant light since day 2 and subjected to sham ECS between days 10 and 22, at 12 h after the original "lights on" time (x).

	DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
(a)					
	2-8 (7)	L:D	100 ± 3.3%	8.00 (12.25)	24.00
CONTROL	9-15 (7)	L:D		(4.00)(8.00)	23.75
	16-19 (4)	L:D	125 ± 4.3%***	(8.00)	23.00
	2-8 (7)	L:D	100 ± 4.1%	8.00 (12.00)	23.75
ECS	9-15 (7)	L:D		(8.00)	23.50
	16-19 (4)	L:D	90 ± 2.4%		23.00
(b)					
	2-8 (7)	L:D	100 ± 3.5%	(8.00)	24.00
CONTROL	9-15 (7)	L:D		(8.00)	23.75
	16-19 (4)	L:D	94 ± 4.3%		23.00
	2-8 (7)	L:D	100 ± 3.2%	(8.00)	24.00
ECS	9-15 (7)	L:D		(8.00)	23.75
	16-19 (4)	L:D	106 ± 2.2%		23.00

Table 8.1 The effects of ECS on locomotor activity of rats, maintained on a normal L:D cycle. Repeated ECS was administered between days 2-14 at (a) 2 h or (b) $9^1/_2$ h after lights on.

The first two columns of the table specify the period in days, for which calculation of mean daily activity counts and spectral analysis of the rhythm were made. The third column indicates the light-dark conditions for each analyzed period. The fourth column contains the mean daily activity expressed as a percentage of mean daily activity during pretreatment period (except for Tables 8.1 and 8.2). The final column depicts the main and secondary (in parentheses) peaks as identified in the spectral analysis records.

*: 0.05, **: 0.02, ***: 0.01, ****: 0.002, *****: 0.001, (Student's t-test), compared to pretreatment period (100%).

		DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
(a)						
		1-8 (8)	L:D	100 ± 7.6%	(8.00) (8.00)	23.75
	CONTROL	9-16 (8)	L:D			23.75
		16-20 (5)	L:D	99 ± 9.4%		23.25
		1-8 (8)	L:D	100 ± 4.5%		23.50
	ECS	9-16 (8)	L:D		(8.00)	23.75
		16-20 (5)	L:D	87 ± 2.6%		23.50
(b)						
		3-10 (8)	L:D	100 ±10.5%	(6.00) (8.00)	23.75
	CONTROL	10-17 (8)	L:D		(8.00)	23.50
		17-20 (4)	L:D	110 ±10.5%	(8.00)	23.25
		3-10 (8)	L:D	100 ± 4.1%		23.75
	ECS	10-17 (8)	L:D		(8.00)	23.75
		17-20 (4)	L:D	78 ± 4.1%*	(8.00)	23.25

Table 8.2 The effects of ECS on locomotor activity of mice, maintained on a normal L:D cycle. Repeated ECS was administered between days 3-15 at (a) 7 h or (b) 12 h after lights on. Details as in Table 8.1.

		DAYS	(n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS		
(a)		1-4	(4)	L:D	102 + 4.3%			
		4-17	(14)	D:L	100 ± 2.9%		(12.00)	25.00
	CONTROL	18-31	(14)	D:L	131 ± 3.1%*****	(8.00)		24.25
		30-37	(7)	D:L	135 ± 1.6%*****	(8.00)		24.00
		1-4	(4)	L:D	86 ± 1.1%			
		4-17	(14)	D:L	100 ± 4.3%	(8.00)	(12.00)	25.00
	ECS	18-31	(14)	D:L	150 ± 4.1%***			24.25
		30-37	(7)	D:L	110 ± 4.1%			24.00
(b)		1-4	(4)	L:D	82 ± 2.3%***			
		4-17	(14)	D:L	100 ± 2.9%			24.50
	CONTROL	18-31	(14)	D:L	132 ± 3.0%*****	(8.00)		24.00
		30-37	(7)	D:L	139 ± 4.9%*****	(8.00)		24.25
		1-4	(4)	L:D	81 ± 4.0%*			
		4-17	(14)	D:L	100 ± 4.4%			24.50
	ECS	18-31	(14)	D:L	157 ± 6.8%*****	(8.00)		24.00
		30-37	(7)	D:L	147 ± 4.2%*****			24.25

Table 8.3 The effects of ECS on locomotor activity of rats, maintained on a reversed L:D cycle. Repeated ECS was administered between days 18-30 at (a) 17 h or (b) 21 h after lights on. Details as in Table 8.1.

	DAYS	(n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
	8-12	(5)	D:L	100 ±19.7%		24.25
	13-20	(8)	D:L	85 ± 5.2%		24.25
CONTROL	21-28	(8)	D:L			24.00
	29-36	(8)	D:L	81 ± 3.4%		24.25
	37-46	(10)	D:L			24.00
	8-12	(5)	D:L	100 ± 1.3%	(8.00)	24.00
	13-20	(8)	D:L	69 ± 3.1%***	(8.00)	24.25
ECS	21-28	(8)	D:L		(8.00)	24.00
	29-36	(8)	D:L	61 ± 2.3%*****	(8.00)	24.25
	37-46	(10)	D:L			24.00

Table 8.4 The effects of ECS on locomotor activity of mice, maintained on a reversed L:D cycle. Repeated ECS was administered between days 13-25 at 16 h after lights on. Details as in Table 8.1.

	DAYS	(n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
CONTROL	24-27	(5)	L:D	35 ± 7.4%*****	(4.00)(4.75)8.00 12.50	23.00
	28-41	(20)	D:D	100 ± 4.3%	8.00 (12.00)	24.00
	34-47		D:D		(8.00)	24.00
	48-61	(14)	D:D	108 ± 3.3%	(4.00)6.00(8.00)(12.00)	23.75
	62-65	(4)	D:D	101 ± 7.7%	(4.75) 6.00 (17.75)	23.25
	66-69	(4)	L:D	91 ± 9.2%		
ECS	24-27	(5)	L:D	41 ±12.8%*****	(4.00)(4.75)8.00(9.00)	23.00
	28-41	(20)	D:D	100 ± 5.2%	(6.00)	24.00
	34-47		D:D		(6.00)	24.00
	48-61	(14)	D:D	139 ± 6.9%*****	(2.25)4.00(6.00)(11.75)	24.00
	62-65	(4)	D:D	138 ± 9.2%***	(6.00)	22.50
	66-69	(4)	L:D	91 ±10.3%		

Table 8.5a The effects of ECS on locomotor activity in rats, freerunning in the dark since day 28. Repeated ECS was administered between days 48-60 at $2^1/_2$h after original lights-on time. Details as in Table 8.1.

	DAYS	(n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS
CONTROL	24-27	(5)	L:D	60 ± 11%**	4.75 (12.00) 23.25
	2-41	(20)	D:D	100 ± 6.0%	24.00
	34-47		D:D		24.00
	48-61	(14)	D:D	82 ± 6.8%	(21.75) 24.25
	62-65	(4)	D:D	86 ±12.6%	(2.00)(3.50)6.00(10.25) 3.25 4.75 7.75 12.00 23.00
	66-69	(4)	L:D	63 ± 5.9%**	
ECS	24-27	(5)	L:D	54 ±20.9%	(2.25)(4.00)(6.00)(10.25) (2.75) 4.75 8.00 23.75
	28-41	(20)	D:D	100 ± 5.3%	(4.50)(8.00)(12.00) 24.00
	34-47		D:D		(4.00)(8.00) 24.00
	48-61	(14)	D:D	107 ± 5.9%	(3.50)4.00(6.00)(21.75) 24.00 (27.00)
	62-65	(4)	D:D	106 ± 11%	(2.00)(3.50) 6.25(23.25) (3.00) 4.25 (11.50)
	66-69	(4)	L:D	70 ± 3.8%*	

Table 8.5b The effects of ECS on locomotor activity in rats, freerunning in the dark since day 28. Repeated ECS was administered between days 48-60 at $9^1/_2$ h after original lights-on time. Details in Table 8.1.

	DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS		
	9-22 (14)	D:D	100 ± 6.8%	7.75	(11.75)	23.00
CONTROL	23-36 (14)	D:D	130 ± 9.3%*		(11.75)	23.50
	37-47 (11)	D:D	109 ± 8.2%		(11.75)	23.75
	9-22 (14)	D:D	100 ±12.8%			23.25
ECS	23-36 (14)	D:D	67 ±7.8%*	(2.25)	23.75	(26.25)
	37-47 (11)	D:D	111 ± 7.5%	(7.75)		23.50

Table 8.6 The effect of ECS on locomotor activity of mice, freerunning in the dark since day 3. Repeated ECS was administerd between days 23-35 at $4\frac{1}{2}$h after original lights-on time. Details as in Table 8.1.

	DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
CONTROL	1-7 (7)	L:D	46 ± 2.4%*****		24.00
	8-14 (7)	L:L	100 ± 5.7%		24.75
	15-21 (7)	L:L		(3.75)(5.75)	25.25
	22-28 (7)	L:L	134 ± 4.3%*****	(2.50)(5.25)(5.75)	25.50
	29-35 (7)	L:L		(4.50)6.00(8.25) 5.25(7.25)	25.00
	36-42 (7)	L:L	137 ±12.7%***	5.25 6.00	25.50
ECS	1-7 (7)	L:D	84 ± 6.3%**	(4.00)(4.75)(8.00)	23.50
	8-14 (7)	L:L	100 ± 3.0%		24.75
	15-21 (7)	L:L		(4.25)(5.00)	25.25
	22-28 (7)	L:L	98 ± 3.1%	(2.00)(3.00)(4.50)(5.00)	25.00
	29-35 (7)	L:L		(2.75)	24.50
	36-42 (7)	L:L	96 ± 2.6%	(6.00)	25.50

Table 8.7a The effects of ECS on locomotor activity in rats, freerunning in the light since day 7. Repeated ECS was administered between days 22-34 at $3\frac{1}{2}$h after original lights-on time. Details in Table 8.1.

	DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
CONTROL	1-7 (7)	L:D	79 ± 5.6%***	(8.00)	23.50
	8-14 (7)	L:L	100 ± 3.0%		24.25
	15-21 (7)	L:L		(3.00)(4.50)	25.25
	22-28 (7)	L:L	103 ± 3.3%	(3.25) 5.25	25.00
	29-35 (7)	L:L		4.75 5.25(6.50)(7.50)	(25.75)
	36-42 (7)	L:L	104 ± 6.2%	(3.00) 4.25 6.00 (3.50) 5.50	25.50
ECS	1-7 (7)	L:D	52 ± 5.7%***	(8.00)	23.50
	8-14 (7)	L:L	100 ± 1.9%		25.25
	15-21 (7)	L:L			25.25
	22-28 (7)	L:L	91 ± 5.1%	(3.75)	25.25
	29-35 (7)	L:L		(1.75)(5.00)(7.00) 3.50 (5.50)(13.50)	24.75
	36-42 (7)	L:L	60 ± 2.7%*****		25.00

Table 8.7b The effects of ECS on locomotor activity of rats, freerunning in the light since day 7. Repeated ECS was administered between days 22-34 at $9\frac{1}{2}$h after original lights-on time. Details as in Table 8.1.

		DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
(a)		3-7 (9)	L:L	100 ±16.4%	(5.00)(7.00) 9.50 19.00 (6.25)(8.50)12.50	25.50
	CONTROL	8-21 (14)	L:L	265 ±24.6%****	(12.50)	25.00
		22-35 (14)	L:L	450 ±24.5%*****		25.25
		3-7 (9)	L:L	100 ±18.4%	12.50(14.50)(18.75)	25.00
	ECS	8-21 (14)	L:L	44 ± 8.4%**	(6.25)(10.00)(17.00)	25.00
		22-35 (14)	L:L	74 ± 8.5%	(8.25)	25.00
(b)		3-9 (7)	L:L	100 ± 32%	(4.00)(12.25)	25.00
	CONTROL	10-23 (14)	L:L	111 ± 6.6%	(8.50)(12.75)	25.25
		24-35 (12)	L:L	266 + 23%****	(8.75)13.00	26.50
		3-9 (7)	L:L	100 ± 14.7%	(12.75)(13.75)(16.00)	25.25
	ECS	10-23 (14)	L:L	71 ± 8.0%	(13.00) (23.50)	26.25
		24-35 (12)	L:L	116 ±11.4%	(11.75) 13.25 (18.50) (12.75)(16.50)	26.50

Table 8.8 The effects of ECS on locomotor activity of mice, freerunning in the light since day 2. Repeated ECS was administered between days 8-20 at (a) 5 h or (b) 12 h after original lights-on time. Details as in Table 8.1.

	DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
CONTROL	4-15 (12)	L:L	100 ± 12%	(12.75)	25.75
	16-29 (14)	L:L	125 ± 8.9%	(4.75)(8.50)(17.25) (7.00)13.00 (18.50)	26.25 (29.75)
	30-34 (9)	L:L	99 ± 8.0%	(6.25)(8.00)(13.25)	26.50
PAROXETINE	5-10 (6)	L:L	100 ± 33%		25.75
	11-22 (12)	L:L	249 ± 26%***	(24.75)	28.00
	23-34 (12)	L:L	327 ±33.5%*****		27.00
MIANSERIN	5-10 (6)	L:L	100 ±19.7%	8.75(13.25)	25.75
	11-22 (6)	L:L	182 ±17.3%**	(8.50)(12.75)	25.50
	23-34 (12)	L:L	177 ± 10.8%****	(13.00)	26.00

Table 8.9 Chronic administration of paroxetine or mianserin in drinking water of mice between days 11-22. Animals freerunning in the light since day 4. Details as in Table 8.1.

	DAYS (n)	L:D	DAILY ACTIVITY COUNTS(%)	PREDOMINANT PERIODS	
CONTROL	8-19	L:L	100 ±10.3%	(8.50)(12.75)	25.50
	20-31	L:L	125 ± 7.2%*	(13.00)	25.50
	25-36	L:L		(13.00)	26.25
	37-48	L:D	285 ±11.8%*****		24.75
TRYPTOPHAN	8-19	L:L	100 ±15.7%	(4.25)(8.50)(13.00) (8.00)(9.00)	26.00
	20-31	L:L	143 ± 17%	(7.75)(13.00)	26.00
	25-36	L:L		(13.25)	26.75
	37-48	L:D	370 ±26.5%*****		24.75(28.50)
CLOMIPRAMINE	8-19	L:L	100 ±10.3%	(8.50)(12.75)	25.50
	20-31	L:L	71 ± 3.6%*****	(2.50)(4.00)	25.25
	25-36	L:L			(22.50)25.00
	37-48	L:D	104 ± 6.4%	3.00	24.50
IMIPRAMINE	8-19	L:L	100 ±21.6%		25.00
	20-31	L:L	316 ± 72%*	(14 peaks)	
	25-36	L:L		(6.75)(11.75) 15.00 21.50 (9.25)(13.00)(17.50)	26.00
	37-48	L:D	105 ± 10%	(3.00)(5.50)(12.25) (3.75)(8.95)(13.50)	24.00

Table 8.10 Chronic administration of tryptophan, clomipramine or imipramine in drinking water of mice between days 20-34. Animals freerunning in the light since day 3. Details as in Table 8.1.

activity, like the control group. The activity of the clomipramine group also showed a modest but significant increase to pretreatment levels whereas the imipramine group retained one third of its activity under the influence of the drug, thus also returning to pretreatment levels (Table 8.10).

As regards the frequencies of the rhythms of the four groups, the control, tryptophan and clomipramine groups all had a freerunning rhythm of between 25.00 and 26.75 hours, the latter having the faster cycle. Upon re-entrainment, rhythm periods verged towards the 24.75 - 24.50 hour values.

The clomipramine group, on the the other hand, presented more problems in interpretation. Although visual inspection of the actogram would exclude any deviation from a normal freerunning rhythm, spectral analysis revealed a very obscure pattern with 14 peaks during the first period of drug treatment, following which, the rhythm freerun with 3 main and several secondary periods. The transition to normal L:D switched the rhythm back to 24.00 hours but still left many secondary peaks.

Overall, tryptophan and clomipramine did not alter the pattern of the freerunning rhythm in mice, whereas imipramine produced a partial breakdown. Clomipramine and imipramine increased and decreased, respectively, locomotor activity. Finally, none of the drugs used hindered the re-entrainment of the rhythm to normal L:D conditions.

8.4. Discussion

The actograms presented in the previous pages display some of the most characteristic properties of a true circadian rhythm. Under

normal conditions, it is entrained to the L:D cycle. If the latter changes, the rhythm can easily re-entrain, in a matter of days to the new schedule. When, finally, constant conditions are imposed (L:L or D:D) the rhythm freeruns with a frequency most probably regulated by the intrinsic pacemaker and, possibly, other contributing environmental factors, that are not easily identifiable. In some ways such a rhythm offers an ideal working model.

It is easy to understand why much hope was resting on the possible effects of ECS on the locomotor activity rhythm, if we consider again the evidence that pharmacological drug treatments slow circadian rhythms of activity (section 3.6.1). A demonstration that ECS, too, could alter the frequency of the rhythm would offer a lot of ground for constructive comparisons between the two different treatments of affective disorders.

Unfortunately, in that sense, the results did not live up to expectations in that the predominant finding of these experiments was one of resistance to repeated ECS of the circadian rhythm of activity in both rats and mice. .

When ECS was administered during the active (dark) phase of the entrained rhythm no effects were observed. This may well be indicative of the stability of an organism, synchronized to its environment. If any kind of disruption had occurred, it would have meant that, administering ECT during the active (light) phase of humans might expose them to changes of unpredictable direction in circadian organization.

A similar lack of effect was observed also when ECS was given during the inactive (light) phase in entrained rats and mice. The

repeated disturbance of the animals could be expected to have at least one consequence: it could cause anticipatory activity, developing after the first few treatments at the expected treatment time and possibly persisting, temporarily, after the termination of treatment. It could, therefore, contribute to the frequency spectrum as a secodary peak. Of course, such an effect would not be specific to ECS-treated animals since it would be the result of handling procedures. As it happens, anticipatory activity was occasionally observed in actograms, mainly of mice, possibly indicating that the handling procedures could not act as a potent zeitgeber, to which the animals might entrain. The results from spectral analysis were in agreement with the findings from actograms. Anticipatory activity has been shown to develop in both entrained and freerunning conditions in various situations as, for example, during restricted feeding (Stephan, 1986a). The value of meal presentation as an effective zeitgeber most probably exceeds the capacity of ECS or handling in that respect.

The use of constant darkness offered the chance to study a species difference. Whilst mice clearly freeran with a rhyhtm of decreased period, rats displayed a rhythm that was marginally slower than 24 hours. Morever. mice in groups easily developed a freerunning rhythm. When groups of rats were used, however, the pattern obtained was very confusing and necessitated the use of isolated animal in order to obtain a freerunning rhythm. Since the same observation was made in four groups of rats and four groups of mice, it is tempting to speculate that the intrinsic period of rats is much closer to 24 hours than that of mice, although the same results might indicate that the pacemaker of rats in the dark is

uncoupled with much more difficulty from the entraining conditions.

No such differences were found when the animals, mice and rats, were allowed to freerun in constant light. There was always a clearly freerunning rhythm with a period increasing by 1 to 2 hours, to 25-26 hours per cycle.

Repeated ECS was also administered to mice or rats under conditions of constant dark or light so that different stages of the freerunning rhythm were examined for an ECS-sensitive phase. The experiments were repeated with administration of ECS corresponding to different points of the rest-activity cycle. The outcome of these experiments does not support the hypothesis that ECS can intervene in the organization of circadian rhythms. The findings were limited to evidence that repeated ECS may reduce locomotor activity during the treatment phase. More specifically, in some cases the activity fell characteristically after the second or third treatment increasing thereafter to pretreatment levels.

The increase in activity seen occasionally during the handling period of the controls might be simply a result of handling or halothane. If this hypothesis is correct, the decreased activity after ECS might be of a higher degree, if it is corrected for the enhancement caused by the procedures.

Even if the effects on locomotor activity were more persistent and clear, ECS was not found to affect the frequency of the rhythms, which was the main target. The reasons for failing to reach such a conclusion are numerous. For example, what has so far been shown is that, when administered at those particular time points, ECS does not affect freerunning circadian rhythms. However, the possibility that there is a vulnerable phase in a rhythm that would respond to ECS

cannot be excluded. A thorough examination would entail treatment with ECS at hourly intervals over 26 hours and would thus require considerable time and animal sacrifice. Still, the possibility that a vulnerable phase does exist and has clinical importance will be discussed in Chapter 11.

Another argument that can be put forward is that ECS can only act when there is desynchrony between environment and organism. The use of freerunning rhythms aimed exactly at providing a model that deviated from an entrained rhythm; however, a freerunning rhythm is, essentially, in compliance to its environment.

Against this, it may be argued that antidepressant drugs, which were shown to phase-delay the rhythms, did so under freeruning conditions. Clorgyline, imipramine and lithium slowed the wheel-running rhythm in hamsters (Wirz-Justice et al, 1982a). Recently, it was also shown that a single injection of triazolam, at doses well below the ones required to induce a hypnotic effect, phase-advanced the wheel-running activity rhythm in hamsters (Turek and Losee-Olson, 1987). Thus, both a delay and an advance of a freerunning activity rhythm is demonstrable. In the present experiments, paroxetine was found to slow considerably the activity rhythm.

An interesting finding is the reported dissociation of activity rhythms in constant conditions, following treatment with clorgyline or imipramine and clomipramine (Redfern and Martin, unpublished observations). In the experiments reported here imipramine caused a breakdown of the locomotor activity rhythm after 11 days of drug administration but clomipramine did not, possibly because a lower dose was used.

The problem of rhythm breakdown to ultradian components was encountered occasionally under unexpected circumstances. In order to discuss this problem, the nature of locomotor activity must be considered. Gross locomotor activity, as measured in mice and rats, is thought to consist of two components. In preliminary experiment during those studies, employing a method that allowed the actual hourly activity to be calculated, it was seen that a block of activity occupied the first half of the active phase. Then, after an observable decrease, a second bout of activity would develop, dominating the last quarter of the active phase and occasionally extending into the beginning of the inactive phase (results not shown).

These two activity components are well documented in the literature and are thought to be dependent on catecholamine (first component) and 5-HT (second component) activity (Hutchins and Rogers, 1973).

Since, as a rule, groups of 3 mice or rats were used, it is not unreasonable to assume that their intrinsic periods are not exactly matching. Thus, three different rhythms, consisting of two components each, contribute to the final pattern, as seen in the actograms. A group of three is the minimum number to constitute a socially interactive group. When one animal is dominant, it can be expected to dictate the pattern of the rhythm obtained in the group, provided that the entraining or freerunning ability of the subordinate animals is such that would allow a synchronization. If none of the animals is dominant, which is improbable, the frequency of the rhythm of each animal may be regulated by the intrinsic pacemaker(s) with minor influences from the cohabitating animals. The latter postulate would explain the breakdown of the pattern.

Evidence from the literature confirms that experimental animals can coexist under constant conditions without synchronizing their rhythm to each other (Kleinknecht, 1985).

A spontaneous breakdown in the activity rhythm has also been reported before, although it followed extensive periods of maintenance under constant light or dark (Eastman et al, 1984), whereas in the present experiments a breakdown in control animals has been observed within 3 to 5 weeks from establishment of freerunning conditions. Wirz-Justice and Campbell (1982) considered the splitting of wheel running activity as a "normal, though infrequent response facilitated by drug treatment".

Since locomotor activity, as measured here, is the sum of a variety of behaviours with an unknown number of individual components, it should come as no surprise when splitting or even breakdown occurs. In retrospect, a group of animals with a disrupted activity pattern might have offered an interesting target on which to apply ECS.

The experimental design also does not allow the evaluation of effects on specific neurotransmitter system. When the locomotor response to apomorphine is measured after repeated ECS an enhancement was reported that was attributed to increased noradrenaline and dopamine activity (Ehlers, Indik, Koob and Bloom, 1983). Earlier, Modigh (1975) had also reported enhanced locomotor response to apomorphine and/or clonidine in reserpinized rats after repeated ECS as a result of increased postsynaptic sensitivity of catecholamine receptors. Although the results of Modigh (1975) included also an estimated increase

in ECS-only treated animals, the experimental design allowed several factors to compound the end effect and should thus not be considered as conclusive evidence.

A final topic to be discussed here is the REM and NREM sleep distribution and its modification by antidepressant therapies. During normal L:D conditions, animal activity, as defined by wheel-running or other activity measurements is not representative of the sleep-wake cycle of the animals. Thus, REM and NREM sleep is observed also during the active phase as, indeed, activity bouts are found during the inactive phase (Mitler et al, 1977). Sleep-wakefulness and activity rhythms did not change in phase when the animals were transferred from normal L:D conditions to L:L or D:D, a result signifying the existence of two (or more) oscillators driving the two rhythms.

In two separate experiments, amitriptyline and nomifensine altered the relative distribution of REM, NREM sleep and wakefulness, an effect varying with the administered dose and possibly other factors such as the recording intervals (Lelkes, Obál, Benedek, Rubicsek, Alfoldi and Obál, 1987; Obál, Benedek, Lelkes and Obál, 1985). Neither drug could, however alter the circadian rhythm of the two sleep variables.

Administration of ECS to cats during an REM sleep deprivation period led to significantly less rebound REM sleep post-treatment, compared to controls. It also decreased REM sleep in normally sleeping cats (Mendels, Van de Castle and Hawkins, 1974). Amitriptyline and nomifensine increased NREM sleep and decreased wakefulness whilst effects on REM sleep were variable and inconsistent but probably detrimental (Lelkes et al,

1987; Obal et al, 1985). Studies in humans are, not unexpectedly, fraught with the problems discussed elsewhere. Nevertheless, it appears that ECT increased total sleep, REM sleep periods and duration and decreased REM sleep latency (Mendels et al, 1974).

In conclusion, ECS does not appear to affect the frequency of the freerunning locomotor activity rhythm of mice or rats nor does it disrupt the entrained activity pattern. Since the transition from normal to freerunning conditions is in itself responsible for changes in the relative duration of the active and inactive phases, and the measured rhythm is a summation of a variety of other behavioural rhythms, it is considered that ECS does not affect the gross behavioural pattern, although this cannot exclude effects on specific activity rhythms.

Clearly, further studies should focus on the monitoring of specific behaviours displaying a circadian rhythm. Apart from investigating the effects of ECS on the frequency or amplitude of such a rhythm, the neurotransmitter system(s) responsible for the expression of the behaviour should be identified. Finally the question of whether a particular rhythm is driven by a separate oscillator or is coupled separately to a common oscillator or even is fully synchronized to the rest of the circadian rhythmsof an organism certainly offers an interesting research angle.

CHAPTER 9 THE PASSIVE AVOIDANCE RESPONSE IN MICE, FOLLOWING SINGLE AND REPEATED ECS

9. THE PASSIVE AVOIDANCE RESPONSE IN MICE, FOLLOWING SINGLE AND REPEATED ECS

9.1. AIM

The aims of these experiments were, first, to confirm the existence of a circadian variation in the passive avoidance response of mice and, secondly, to investigate the effect of a single or repeated ECS on the response by altering the time intervals between learning the response, treatment and retesting.

9.2. Materials and Methods

9.2.1. Animals

Male CFLP mice, aged $6^1/_2$ to $9^1/_2$ weeks, on completion of the experiment, and maintained under normal or reversed L:D conditions as described in Chapter 6, were used in groups of 8-10.

9.2.2. ECS

ECS was administered according to the specifications described in section 6.4.

9.2.3. Experimental Apparatus

The passive avoidance apparatus consisted of an open-topped, perspex box, measuring 22.5 x 16.5 x 16 cm. The floor of the box was covered with four metal plates measuring 11x8 cm and placed in such a way that a 4.5 mm gap was left between any two adjacent plates. A perspex cross of the same height (16 cm) was placed so that it fitted in the gap between the plates, dividing the box into four compartments. A small apperture (2.8 x 2.8 cm) was cut off each side of the perspex cross, where it met the walls of the box. This

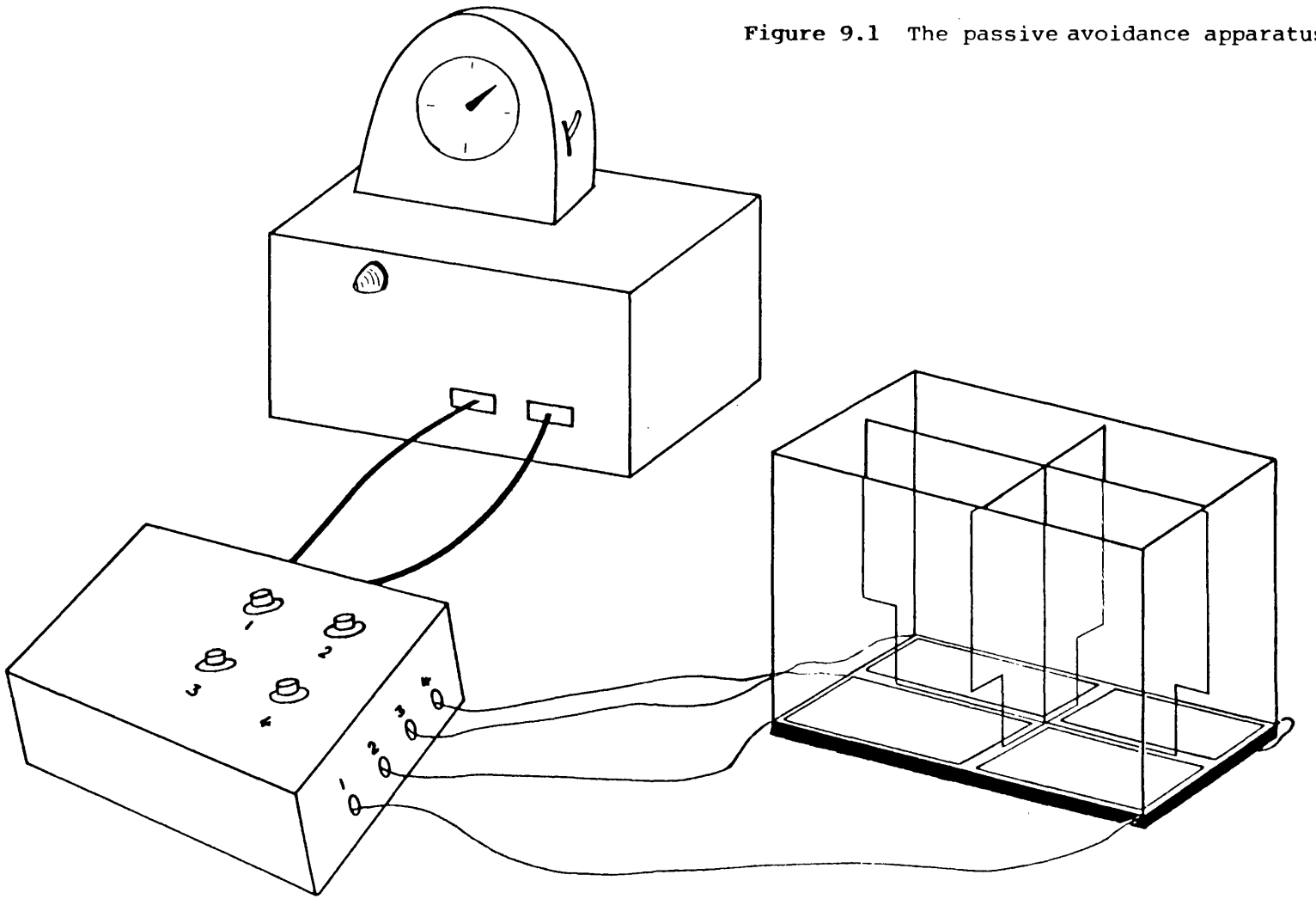


Figure 9.1 The passive avoidance apparatus.

allowed communication between any two adjacent compartments (Fig. 9.1).

The plates were connected to a transformer via a selector panel which allowed the electrification of any chosen plate. The administerd shock was a constant current of 0.2mA and 500V.

The method, without the modification of the perspex cross, has been used for screening minor tranquillizers (Boissier et al, 1968) and, recently, the effects of benzodiazepines on the 24-hour variation in the passive avoidance response was reported (Childs and Redfern, 1981).

9.2.4. Testing Procedures

On the experimental day, the animals were transferred from their usual environment to the experimental room 1 hour before they were used, minimizing any disturbances. On first exposure, each animal was gently transfered in the apparatus and allowed 15" to explore it. For the next 1', each crossing from plate to plate through the appertures was punished with a brief (0.5-1.0s) administration of current to the plate to which the animal was crossing. Crossings were counted for the initial 15" period and each 15" interval of the ensuing 1', by the end of which the mouse was returned to its home cage. In some experiments, ECS was administered at this point. Any animals which made no attempts to cross, and those which crossed more than 20 times were excluded. This process will be referred to as the first session or learning trial.

At different intervals, depending on the nature of the experiment, the mice were reintroduced to the apparatus for the

second trial (second exposure, retest or retention trial). The animals were always placed on the same plate with the same orientation and again allowed a 15" interval before the administration of footshock for any crossing or any attempt involving the front paws and more than 50% of the body, stretched through the apperture to the next plate during 1'. Again, crossings were counted for each 15" period. In one series of experiments, retention was measured without the administration of footshock.

After each individual session the experimental apparatus was cleaned of faeces and urine and after each group it was washed with soap and water, and ethanol.

9.2.5. Time of Experimentation

When a 24-hour variation in the response was examined, the animals were tested for retention exactly 24 hours after their initial exposure. In the dark phase both training and retesting took place under dim red light.

All other experiments took place during the morning hours corresponding to 5 to 8 hours after lights on.

9.2.6. Presentation of Results and Statistical Analyses

The average number of crossings for each group (mean $\pm$ sem) over the one-minute learning session was compared to the score for one minute on retention by the Student's t-test for paired data. The number of crossings for the initial 15 seconds was not taken into account. The scores of control and experimental groups during learning or retention were also compared by the Student's t-test.

Retention was also expressed as:

$$\frac{\text{(1st session score - 2nd session score) x 100}}{\text{1st session score}}$$

Where applicable the variation of the response over 24 hours was examined by one-way analysis of variance (ANOVA).

Finally, the average number of crossings per group per 15', including the initial 15' before footshock was administered, was analyzed by ANOVA.

9.3. Experimental Design and Results

9.3.1. 24-Hour Variation in the Passive Avoidance Response

The passive avoidance response was measured at 9 times points throughout a normal 24-hour cycle, namely at 1,4,7,10,13,15,18,20 and 23 hours after lights on (Fig. 9.2). The first five are points in the light and the following four are in the dark phase of the cycle. Although there was an evident fluctuation, the response both during the learning and retention sessions did not reach statistically significant levels. In contrast, when retention scores were expressed as a percentage of learning scores (retention %, Fig. 9.2) there was a significant variation even though it seemed to be derived from a particularly poor retention observed one hour before lights went out (d.f., 8/77, f-value: 3.23151, $p<0.01$ ANOVA).

In a separate experiment, the same variation was examined with the difference that, during retention, the animals were not subjected to footshock (Fig. 9.3). Here, learning scores just failed to reach statistically significant levels for their variation with time, whilst retention scores showed a marked variation (d.f. 8/78, f-value: 3.95591, $p<0.01$, ANOVA). Surprisingly, despite the evident trend

for retention % to decrease during the dark phase, no significant variation was recorded. Retention scores in the dark phase, when footshock was not administered, were consistently higher than retention scores in the dark phase when footshock was administered.

If the average number of crossings for each 15° interval are combined, irrespective of testing time, the picture that emerges is shown in figures 9.4 and 9.5. On the learning session, the mean number of crossings increased dramatically when footshock was applied, followed by a steep decrease until the end of the experimental minute. In both experiments there is a significant fluctuation (Fig. 9.4: d.f. 4/40, f-value: 16.2391, $p<0.01$, ANOVA; Fig. 9.5, d.f. 4/40, f-value: 11.1388, $p<0.01$, ANOVA). In contrast, on retention the animals that were given a footshock displayed another marked decrease in response (Fig. 9.4: d.f. 4/40, f-value 3.47 $p<0.05$, ANOVA) whereas those without footshock showed a steady response throughout the duration of the test (Fig. 9.5).

9.3.2. The Effects of a Single ECS

The intervals between treatment, training and retesting for the next six experiments involving a single ECS or sham ECS are shown below. The numbers in parentheses denote the relevant figures.

Training —24h→ ECS —1h→ retention (9.6a)

Training —24h→ ECS —6h→ retention (9.6b)

Training —24h→ ECS —24h→ retention (9.6c)

Training —1h→ ECS —24h→ retention (9.7a)

Training —1h→ ECS —1h→ retention (9.7b)

ECS —24h→ Training —24h→ retention (9.7c)

A single ECS applied 24 hours after training did not impair learning of the response as measured by retention one hour after treatment (Fig. 9.6a), decreased it at 24 hours (fig. 9.6c) and, surprisingly, increased it at 6 hours (fig. 9.6b).

When treatment was applied 1 h after training, retention was normal at 1 hour after treatment (Fig. 9.7b) but, again, impaired at 24 hours (fig. 9.7a).

Pretreatment with ECS 24 h before training did not have any effect on retention measured 24 hours after training (9.7c).

As can be seen in Fig. 9.8, when controls are considered as a whole, they show the familiar sharp increase followed by a decline to almost pre-footshock levels, whereas ECS-treated and control animals behave, on retention, similarly to each other and show a continuous decline, although only halothane-treated groups displayed a statistically significant fluctuation over time. Apart from the initial 15", at all other intervals the mean scores at learning were significantly higher than either group; ECS groups differed at only one interval from halothane groups.

9.3.3. Effects of Repeated ECS

For the next six experiments, the intervals between treatment

and learning and retention trials are presented below, with the number of the corresponding figure.

7 x ECS	24h ——→	learning	1h ——→	retention	(9.9a)
7 x ECS	24h ——→	learning	24h ——→	retention	(9.9b)
7 x ECS	24h ——→	learning	5 days ——→	retention	(9.9c)
7 x ECS	5 days ——→	learning	24h ——→	retention	(9.10a)
training	5 days ——→	7 x ECS	24h ——→	retention	(9.10b)
training	24h ——→	7 x ECS	24h ——→	retention	(9.10c)

Following a series of 7 ECS, mice were trained 24 hours after the last treatment. Retention measured 24 hours after learning was normal (fig. 9.9b), but when measured within 1 hour or after 5 days it was impaired (9.9. a and c).

When the interval between the last treatment and learning was increased to 5 days and retention was tested 24 hours after training no evidence of impairment was found (9.10a).

Finally, treatment was interspaced between learning, at 5 days (9.10b) or 1 day (fig. 9.10c) after learning and 1 day before retention. Thus the interval between first and second trial was 19 and 15 days, respectively. In both cases memory was impaired.

Considering the results as a whole, halothane-treated groups

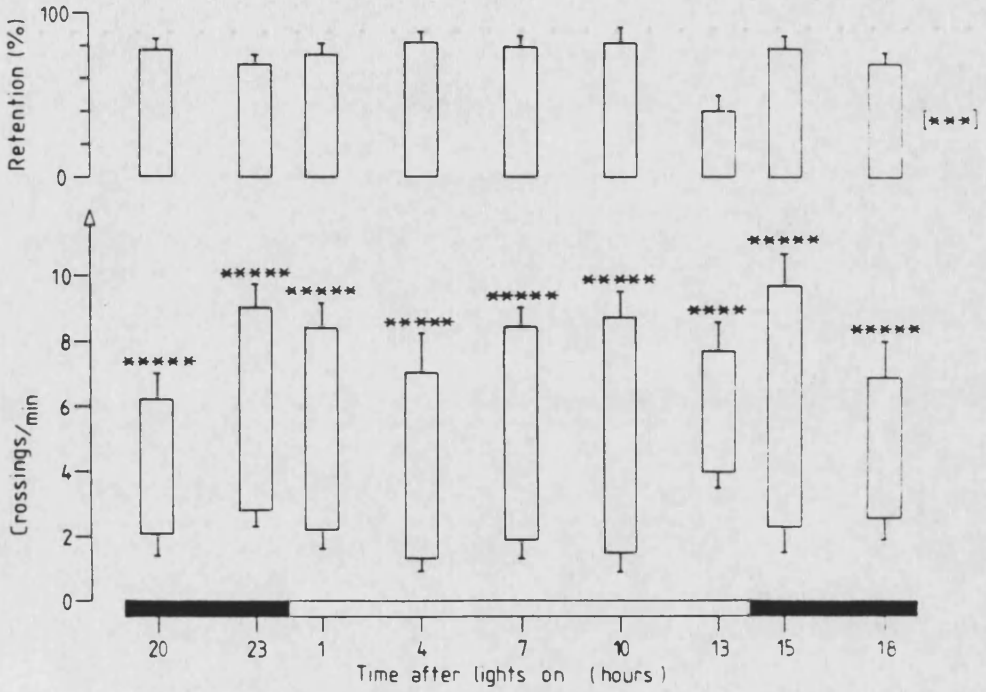


Figure 9.2 The passive avoidance response in mice over 24 hours. Lower section: the top of each bar is the mean score ± s.e.m. for learning, the bottom is mean score ± s.e.m. for retention. Upper section: retention as a percentage (+ s.e.m.) of learning score. Black bar on time axis denotes dark phase of the L:D cycle.

n = 8-10 for each group; ****: $p<0.002$, *****: $p<0.001$ (t-test), compared to retention score; [***] : $p<0.01$ (ANOVA, d.f. 8/77, f: 3.23151).

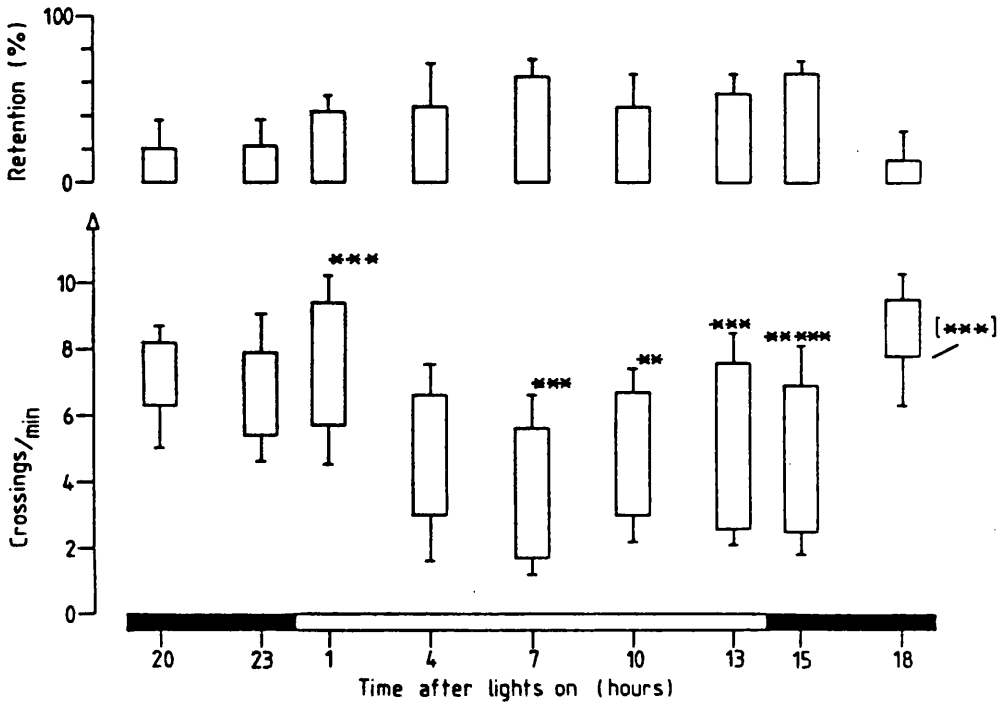


Figure 9.3 The passive avoidance response in mice over 24 hours, with no footshock applied on retention. Lower section: the top of each bar is the mean score ± s.e.m. for learning, the bottom is mean score ± s.e.m. for retention. Upper section: retention as a percentage (± s.e.m.) of learning score. Black bar on time axis denotes dark phase of the L:D cycle.

n=8-10 for each group; **: $p<0.02$, ***: $p<0.01$, *****: $P<0.001$ (t-test), compared to retention score; [***]: $p<0.01$ (ANOVA, d.f. 8/78, f: 3.95591) for retention scores over 24 hours.

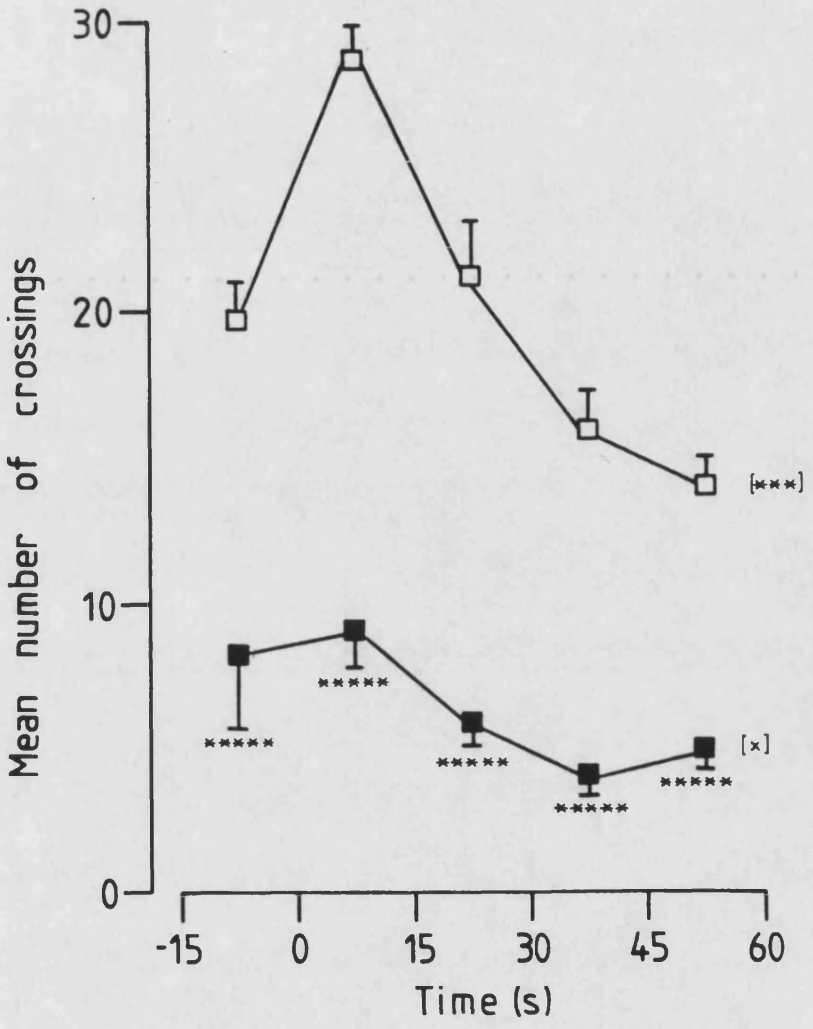


Figure 9.4 Group mean scores per 15" from groups in Fig. 9.2 (footshock applied on retention). White squares: learning. Black squares: retention.

n=9 at each time point. *****: $p<0.001$ (t-test), compared to learning scores; [*]: $p<0.05$ (ANOVA, d.f. 4/40, f: 16.2351); [***]: $p<0.01$ (ANOVA, d.f. 4/40, f:3.47)

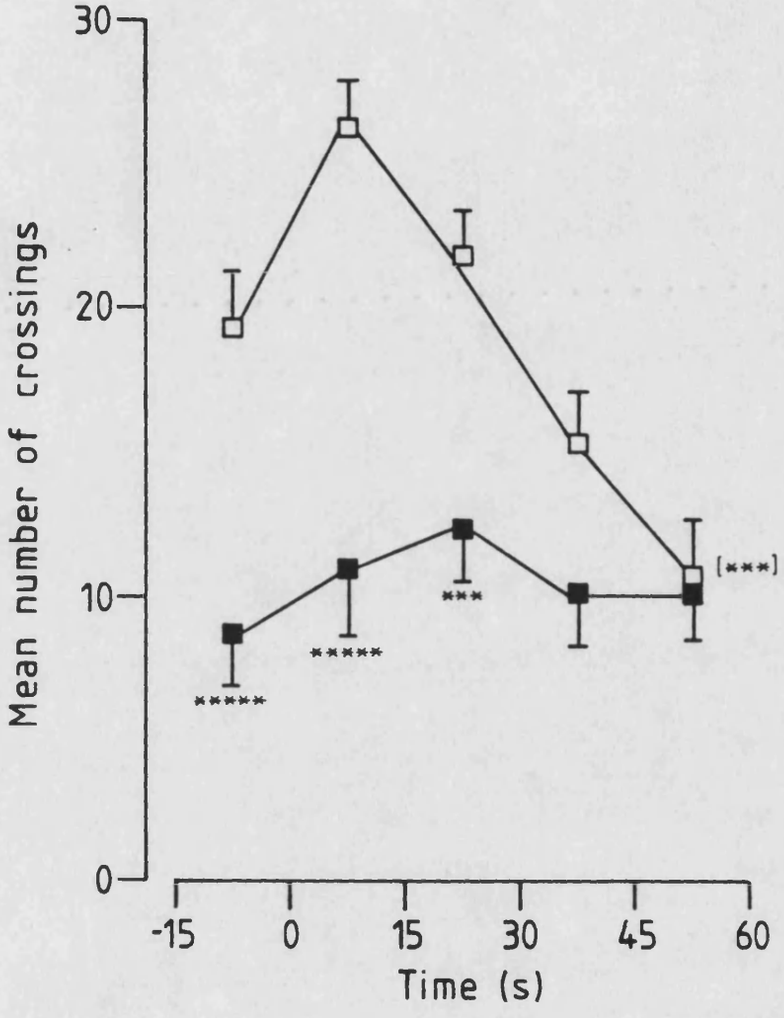


Figure 9.5 Group mean scores per 15" from groups in Fig. 9.3 (footshock not applied on retention). White squares: learning. Black squares: retention.

n=9 at each time point. ***: p<0.01, *****: p<0.001 (t-test), compared to learning score; [***]: p<0.01 (ANOVA, d.f. 4/40, f: 11.1388), for scores on learning.

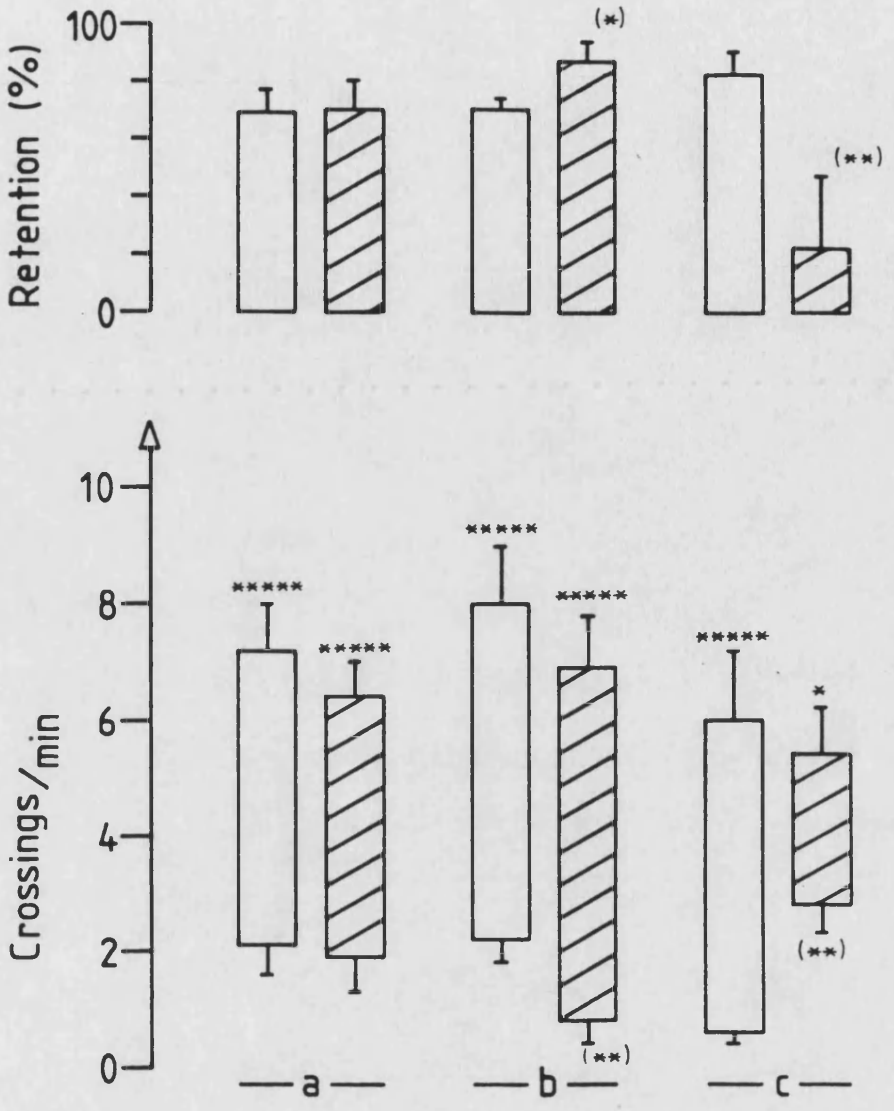


Figure 9.6 The effects of 1 x ECS, applied in mice 24 hours after training, on retention tested (a) 1 h; (b) 6 h or (c) 24 h after treatment. Open bars: control animals; Hatched bars; ECS-treated animals.

n=8-10; mean ± s.e.m.; *****: $p<0.001$ (t-test) compared to retention score of the same group; (*): $p<0.05$, (**): $p<0.02$ (t-test) compared to appropriate control group.

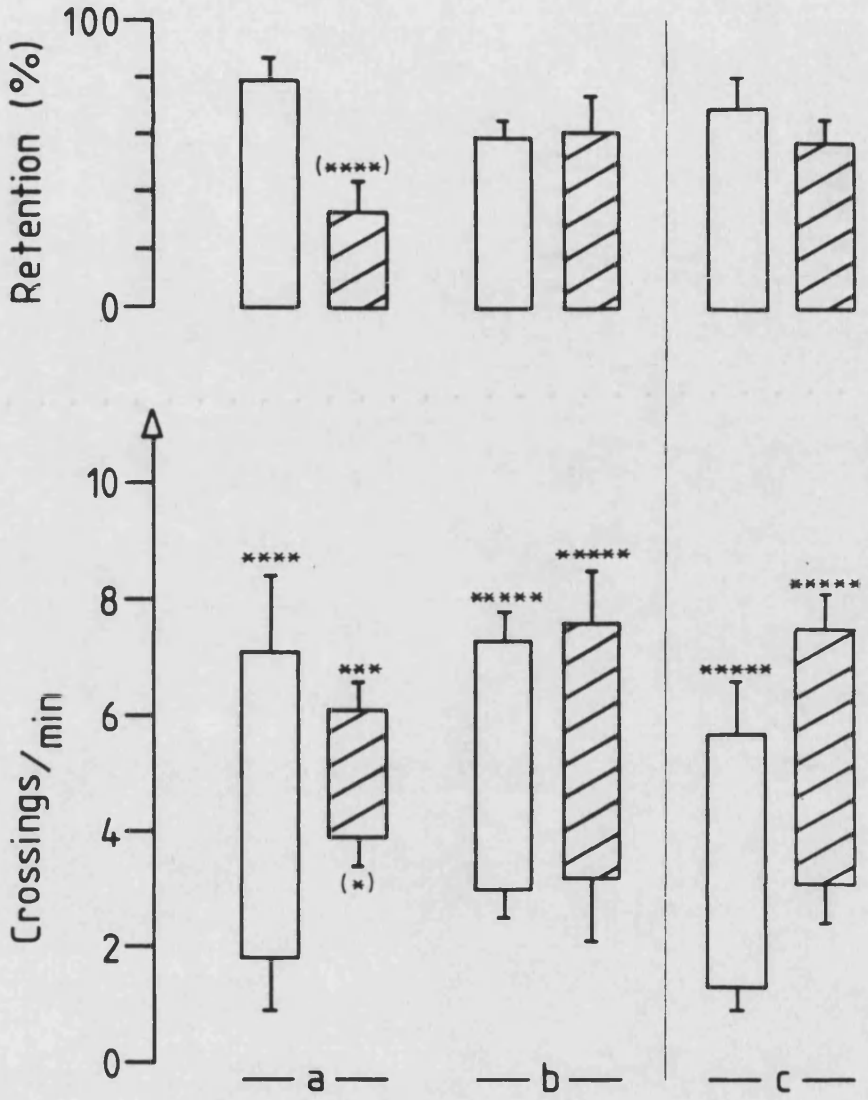


Figure 9.7a,b The effects of 1 x ECS, applied in mice 1 h after learning session, on retention tested (a) 24 h or (b) 1 h after treatment. **Fig. 9.7c** The effect of 1 x ECS, applied 24 h before learning, on retention, tested 24 h later. Open bars: control animals. Hatched bars: ECS-treated animals.

n=8-10; mean ± s.e.m.; ***: $p<0.01$, ****: $p<0.002$, ***** $p<0.001$ (t-test) compared to retention score of the same group; (*): $p<0.05$, (****): $p<0.002$ (t-test), compared to appropriate control group.

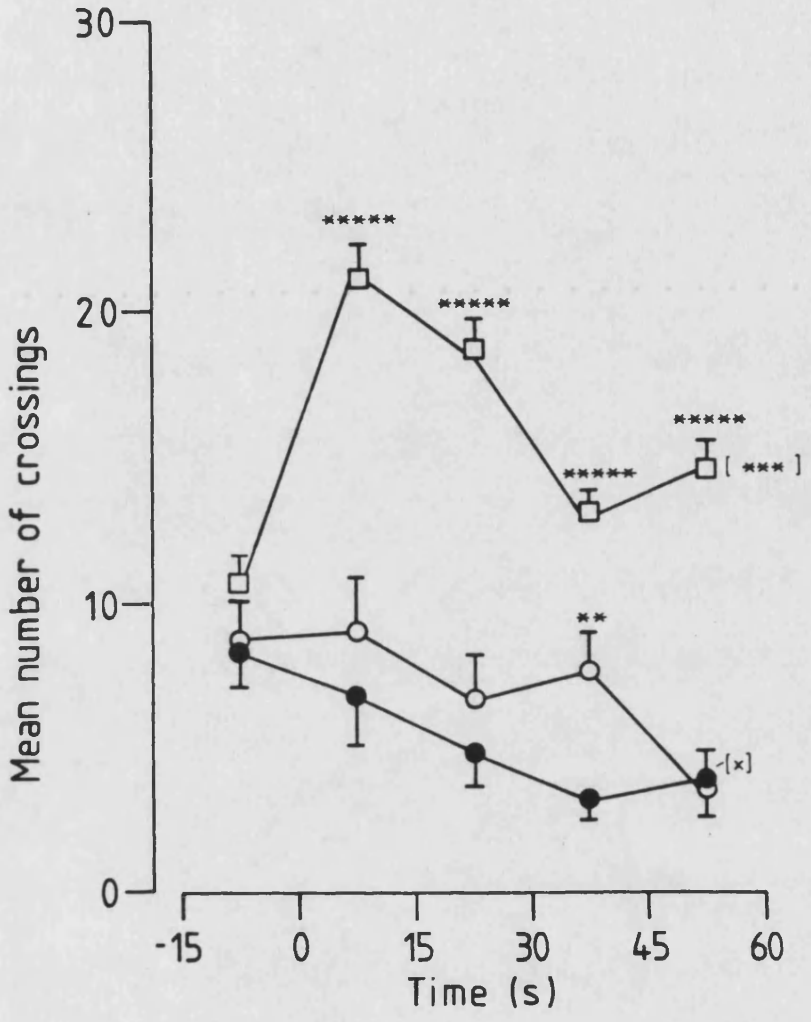


Figure 9.8 Group mean scores per 15" on learning (all groups in Figs. 9.6 and 9.7; white squares, n=12) and retention (control groups, black circles, n=6; 1 x ECS-treated groups, white circles, n=6). **: $p<0.02$, *****: $p<0.001$ (t-test), compared to retention score of controls (black circles); [*]: $p<0.05$ (ANOVA, d.f. 4/55, f: 2.8718); [***]: p 0.01 (ANOVA, d.f. 4/55, f: 17.6308).

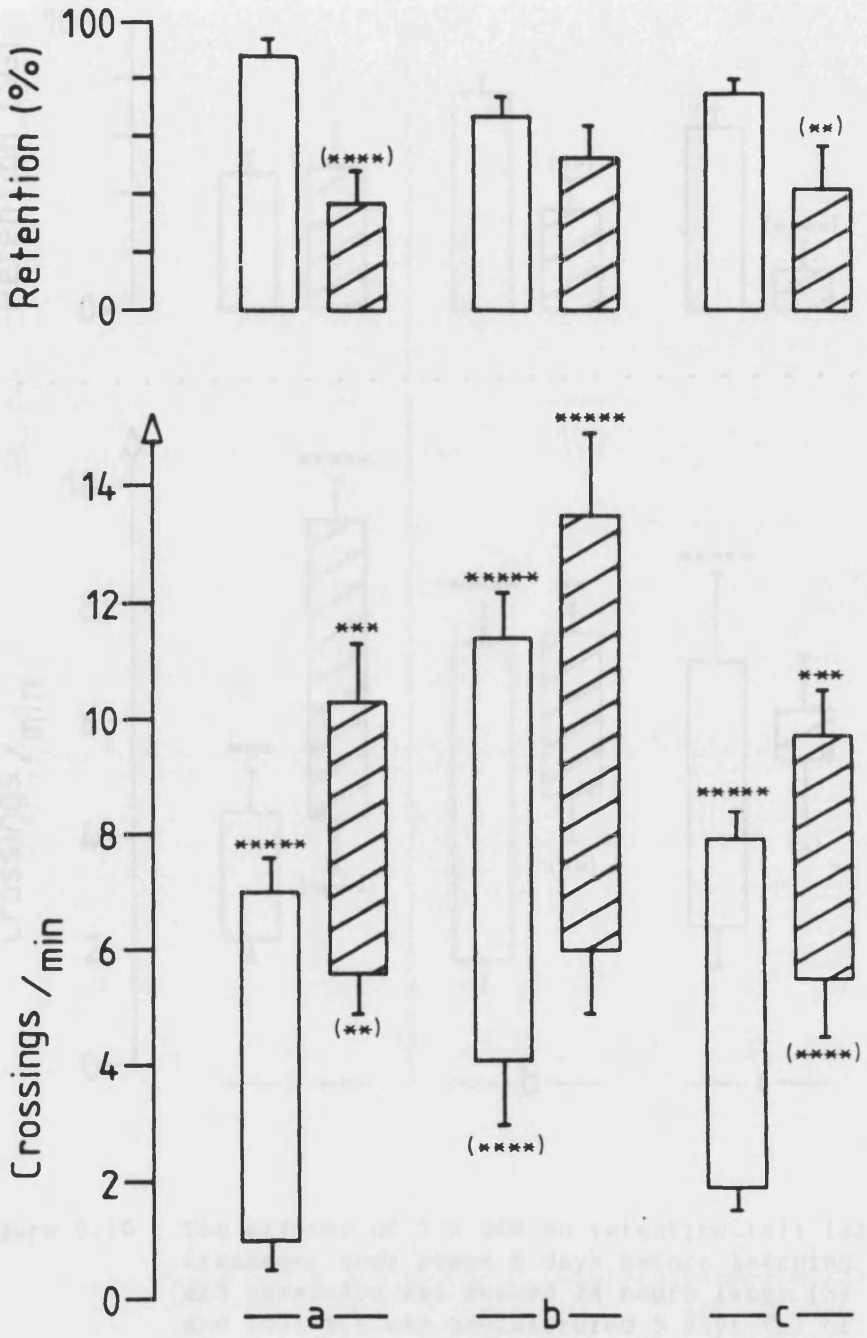


Figure 9.9 The effects of 7 x ECS on retention. Last treatment took place 24 h before learning and retention was tested (a) 1 h, (b) 24h or (c) 5 days after learning. Open bars: control animals. Hatched bars: ECS-treated animals.

n=8-10; mean ± s.e.m.; ***: $p<0.01$, *****: $p<0.001$ (t-test), compared to retention score of the same group; (**): $p<0.02$, (****): $p<0.002$ (t-test), compared to appropriate control group.

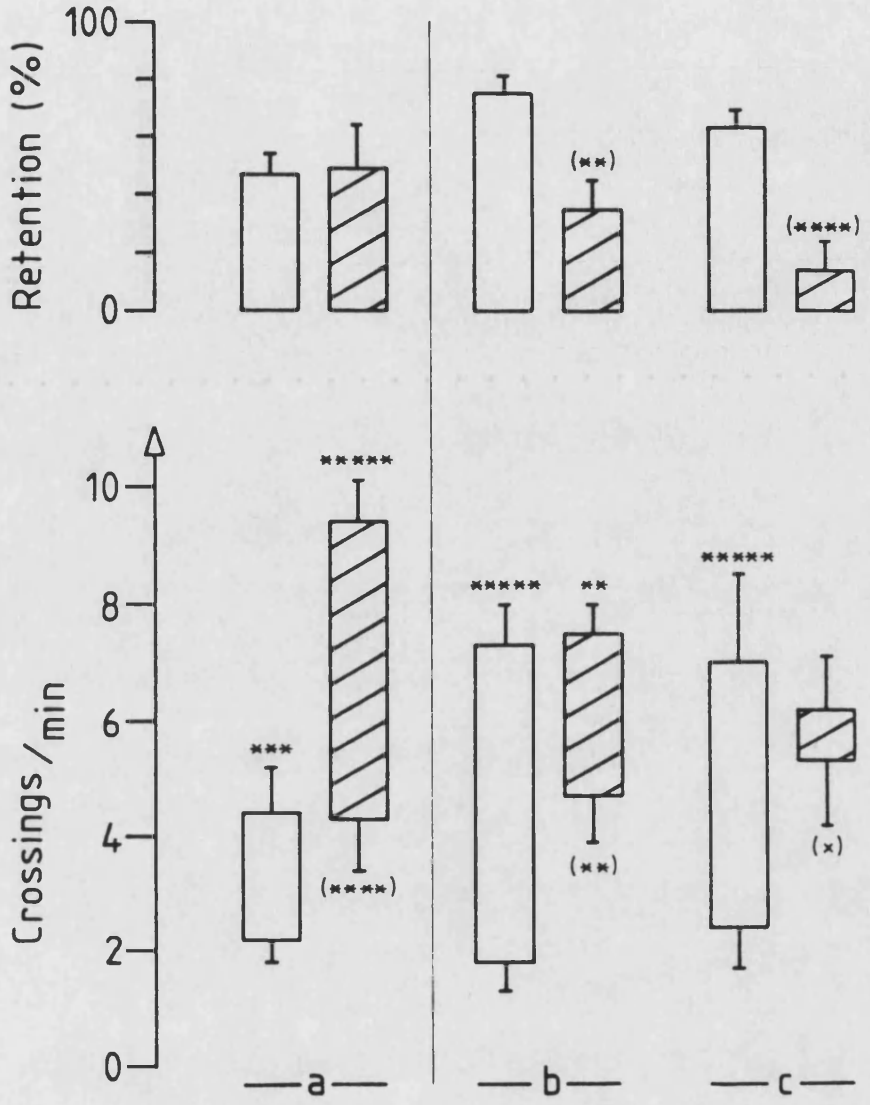


Figure 9.10 The effects of 7 x ECS on retention.(a): last treatment took place 5 days before learning and retention was tested 24 hours later.(b) and (c): ECS was administered 5 days (b) or 24 hours (c) after learning and retention was tested 24 hours after the last ECS.

n=8-10; mean ± s.e.m.; **: $p<0.02$, ***: $p<0.01$, *****: $p<0.001$ (t-test), compared to retention score of the same group; (*): $p<0.05$, (**): $p<0.02$, (****): $p<0.002$ (t-test), compared to appropriate control group.

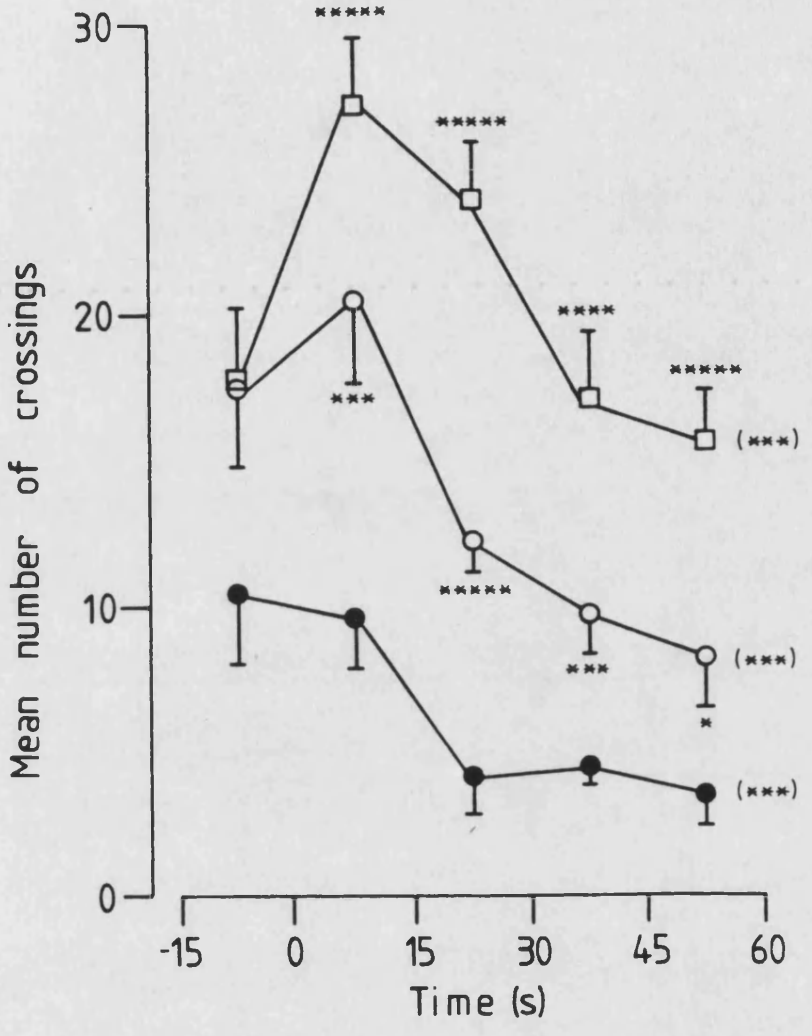


Figure 9.11 Group mean scores per 15" on learning (all groups from Figs. 9.9 and 9.10; white squares, n=12) and retention (control groups, black circles, n=6; 7 x ECS-treated groups, white circles, n=6).

*: $p<0.05$, ***: $p<0.01$, ****: $p<0.002$, *****: $p<0.001$ (t-test), compared to retention score of controls (black circles); [***]: $p<0.01$ (ANOVA, d.f. 4/55, f: 5.05471, white squares; d.f. 4/25, f: 6.18503, 4/25, white circles; and, d.f. 4/25, f:4.2466, black circles).

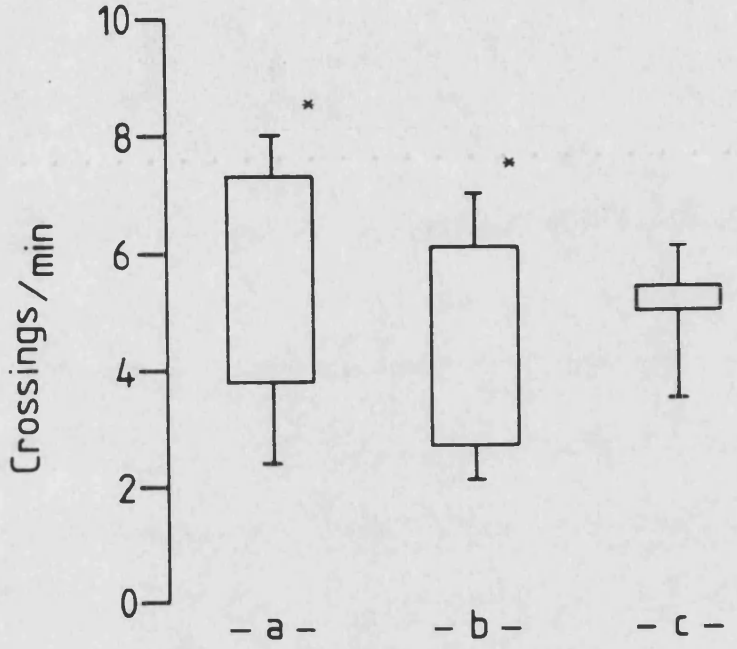


Figure 9.12 The passive avoidance response in mice pretreated with (b) pCPA (300 mg/kg, s.c., once daily for 4 days) or (c) reserpine (3 mg/kg, s.c., 1' after learning) compared to control (a). Retention was tested (b) 14 days or (c) 8 days after treatment. (*): $p<0.05$ (t-test).

showed a marked decrease in activity without an initial surge, as expected, and scores from learning trials were in agreement with the previous experiments (fig. 9.11). However, on retrial, ECS-treated animals showed a pattern that resembled the pattern seen at learning and yet values at each time interval were significantly higher than halothane-treated groups and significantly lower than they were during the first session (fig. 9.11). Learning scores from animals that were pretreated with ECS were not statistically different from those in which learning preceded treatment and thus were combined, in the upper trace of figure 9.11.

9.3.4. Effects of pCPA and Reserpine

Reserpine, 3 mg/kg s.c., was administered once to mice within one minute of learning and retention was tested 8 days later. In another group, pCPA 300 mg/kg s.c., was administered once daily for 4 days. On the fourth day, the animals were subjected to the learning session with retention examined 14 days later. Control animals were injected with vehicle for 4 days or once after the training session. Retention was measured at 8 or 14 days and the average for the controls was taken, since there was no difference between them (7.12a).

Reserpine reduced retention to pretreatment levels (fig. 7.12c) whereas pCPA did not have any measurable effects (fig. 7.12b) compared to control group.

9.4. Discussion

Passive avoidance is a procedure that promotes precisely a passive response to a given stimulus. When the animals are first

introduced to the apparatus they tend to explore it for the first, unpunished 15 seconds. After that, they initially respond to footshock by increased activity (escape behaviour) until they connect the noxious stimulus with crossing from plate to plate, and show a tendency to limit their activity by remaining on any one plate. In the original version of this set-up, i.e. without the perspex cross, it was found that the animals often occupied the centre of the apparatus, being in contact with more than one plate at each moment and giving rise to very obscure and subjective behavioural patterns. Also, the establishment of a link between punishable behaviour and punishment must have been more difficult for the animals, before the modification of the cross was introduced.

During the retention trial, commonly, the animals would display limited activity during the initial 15", possibly because they "recognize" the environment. When footshock is re-established, animals that do not appear to "remember" show increased movements from plate to plate whereas the ones that presumably "remember" use the noxious stimulus as a reminder and dramatically eliminate their activity. Typically, the last category of animals will present increased urination and defecation during their residence in the experimental apparatus.

It has been shown in the past that there is a 24-hour variation in the passive avoidance response of rats under different experimental conditions (Davies, Navaratnam and Redfern, 1973) and mice under very similar conditions to the ones used in this set of experiments (Childs, 1982). However, it was necessary to replicate the finding, firstly because the experimental apparatus was modified

and secondly because it was considered important to obtain a rhythm by testing the animals under such light conditions that correspond to their phase, although Childs (1982) postulated that there was no difference when animals from the dark phase were trained and tested under bright light.

In the present experiments, a variation over 24 hours is also reported although it is not as pronounced as in the evidence of Davies et al (1973) and Childs (1982). It is quite interesting that, when footshock was abolished during the retention session, the responses were markedly more variable during 24 hours. Thus it may be postulated that during the light phase the remembrance of the footshock overcomes the natural tendency to explore, hence the low scores, whereas during the dark phase, the "fear" of punishment is overcome by the naturally increased urge to display locomotor acivity. Under these conditions, the trend for passive avoidance to be more effective during the light phase is in agreement with the aforementioned evidence but puts in doubt the assumption that the reason is enhanced learning and/or retention. In any case, the putative 24-hour variation could not be considered a true circadian rhythm since it has, so far, not been shown whether it meets the appropriate criteria as mentioned in the introduction.

With respect to ECS, it could be said that, overall, a single ECS does not affect the response of the animals. Diminished retention found when ECS was given within 1 hour of learning (fig. 7.6a) points towards effects of ECS on memory consolidation, an assumption reinforced by the detrimental effects of reserpine (fig. 7.11c; also, Dismukes and Rake, 1972). The normal retention found within 1 hour after ECS could be attributed to temporary reduction in

locomotor activity that may follow ECS treatment. In the previous chapter, however, it was shown that there is no consistent reduction in locomotor activity, following ECS.

In an equally general manner, it can be said that repeated ECS affects retention. In terms of retrograde amnesia, repeated ECS produced low retention regardless of the learning-treatment interval. On the other hand, the effect of ECS on anterograde memory are variable: apparently it interfered with learning or consolidation, since retention 1 hour after learning was impaired, and with long-term memory since impairment was seen at 5, but not 1, days after learning.

It would be dangerous to reach any conclusions on the basis of these data. The reason is that more studies are needed in order to examine all the possible intervals beween the two sessions and treatment. However, studies involving learning and retention within hours of treatment may not be critical, since in realistic terms, the events that may precede or follow within hours from each treatment of any hospitalizised patients, during a course of ECT, would be hardly expected to be of vital importance. Thus priority should be given to the effects of ECS on memory for events spaced beyond the time period surrounding each treatment.

While this work was in progress, Lerer et al (1986) published their work which used a different passive avoidance apparatus in rats but a very similar protocol. Their results are essentially opposite to the ones reported here whenever the experimental time intervals are comparable in the two studies, although their final conclusions suggest both anterograde and retrograde amnesia associated with

repeated ECS. Perhaps, a significant difference in the methodology is the fact that Lerer et al (1986) treated their rats with daily ECS, whereas the present schedule is one ECS every second day. It has yet to be examined whether such a difference in schedule could precipitate differences in the effects of ECS on learning and memory.

Another field that this study was intended to cover is the effect of different changes in some of the parameters of ECS (pulse width, frequency) on the passive avoidance response as well as the combination of ECS with drugs that are known to affect memory processes and drugs that are usually prescribed for psychiatric patients, before, during or after the ECT course. This study might realise a wealth of evidence on the mechanism by which ECT affects memory. Also the effects of ECS at different points in the L:D cycle should be investigated, in order to examine the possible effects of ECS on neurotransmitters which are both involved in the learning and memory processes and display a circadian rhythm.

In conclusion, there is evidence suggesting that the passive avoidance response shows a 24 hour variation. A single ECS does not consistently change this response whereas repeated ECS appears to induce both anterograde and retrograde amnesia. The fact that the experiments were conducted at a phase when retention is high, and despite the fact that it is unclear what this improved retention is caused by, acts as a reinforcement for results showing poor retention connected with ECS.

CHAPTER 10 THE HEAD-TWITCH RESPONSE

10. THE HEAD-TWITCH RESPONSE

10.1. Aim

The head-twitch response in mice is a behaviour attributed to the stimulation of 5-HT_2 receptors in the brain (section 1.11.2). The effects of ECS on the response have often been the subject of research in the past. The limited number of experiment presented here aimed at using the effects of ECS on the head-twitch response as a means of demonstrating the efficacy of ECS, as administered in this study.

10.2 Materials and Methods

10.2.1. Induction of the Response

Female mice aged four weeks (chronic studies) or six weeks (acute studies) were used in groups of 10.

The head-twitch response was evoked in two ways:

(a) Carbidopa (25 mg/kg, i.p.) was injected 20 minutes before the administration of 5-HTP, (50-100 mg/kg, i.p.). Twenty minutes later, the animals were observed individually and the number of head-twitches was recorded for three minutes.

(b) Alternatively, mice were injected with 5-MeODMT, 5mg/kg, i.p., one minute before the three-minute observation period (Moser and Redfern, 1985). All observations took place between 14:00 and 17:00 hours, corresponding to the period of 9 to 12 hours after lights on. ECS was administered according to the guidelines laid out in section 6.4 .

The results are expressed as the percentage of the mean head-twitch response compared to the corresponding control $\pm$ s.e.m.

10.2.2. The Head-Twitch Response Following Oral Antidepressant Drug Administration

Three groups of mice were injected with a loading dose of (a) mianserin, 2 mg/kg, i.p.; (b) desmethylimipramine (DMI), 30 mg/kg, i.p.; or (c) 0.2 ml distilled water (control group). For the following 18 days, the three groups had free access to food and water bottles containing, respectively: (a) mianserin solution in distilled H_2O, (b) DMI solution in distilled H_2O and (c) distilled H_2O. Water consumption was measured at 24- or 48-hour intervals and fresh drug solutions were provided. Assuming uniform consumption among the mice and allowing for the increase in body weight, each animal received, on average 2.22 mg/kg/day mianserin or 25.3 mg/kg/day DMI during the experiment.

On the 18th day, the head-twitch response was evoked by 5-HTP (50 mg/kg, i.p.). The animals were then kept on distilled H_2O. Two days later, the same animals were used again to measure the head twitch response following 5-HTP administration.

10.2.3. The Head-Twitch Response Following ECS

The head-twitch response was measured following administration of either 5-HTP (100 mg/kg) or 5-MeODMT in groups of mice treated with (a) a single ECS, three hours prior to observation; (b) a single ECS, 24 hours prior to observation and; (c) a course of 7 ECS administered over 13 days, with observation taking place 48 hours after the last ECS. Control animals were treated with halothane only.

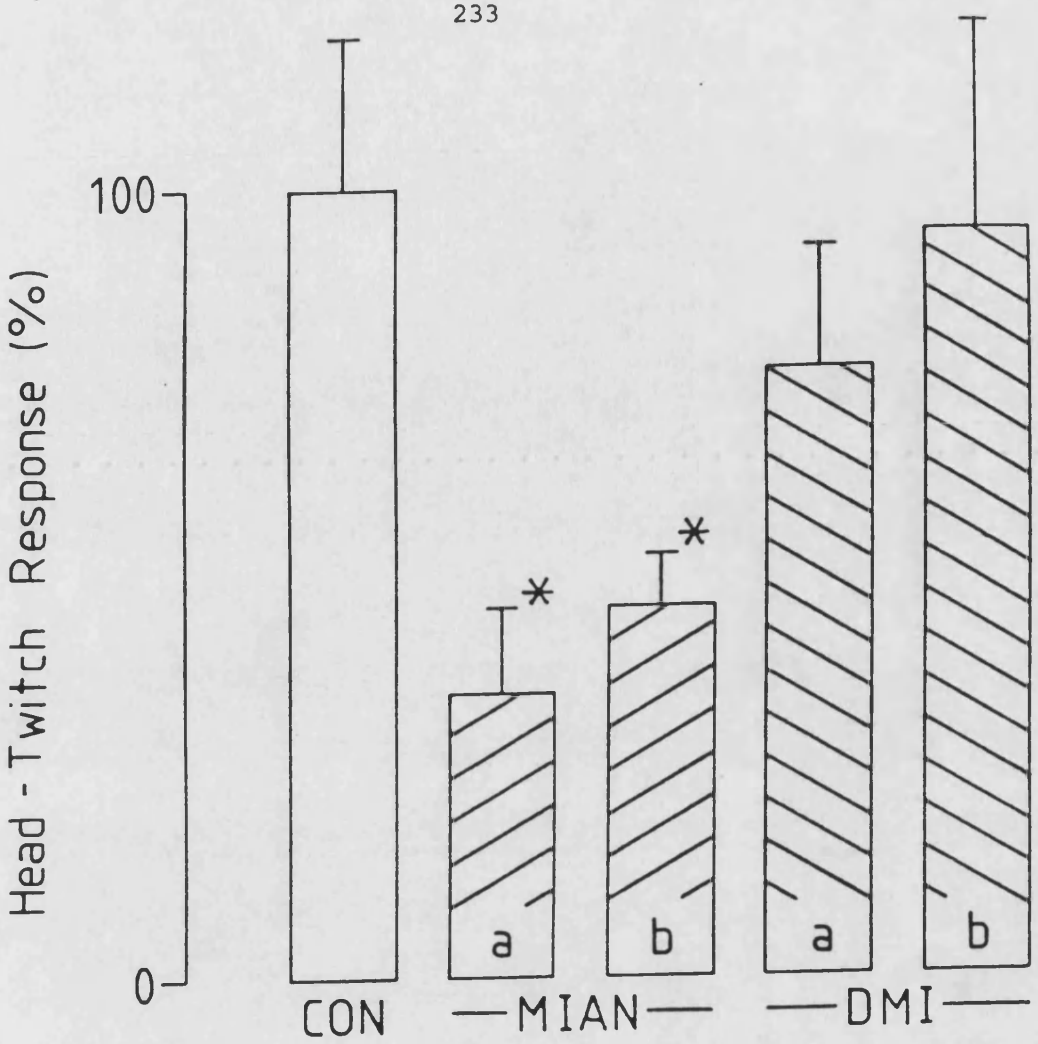


Figure 10.1 Mean head-twitch response evoked by 5-HTP (50 mg/kg, i.p.) in mice, chronically treated with mianserin (MIAN) or desipramine (DMI). (a) Last day of treatment (b) 48 hours after drug withdrawal. *: $p<0.05$ (t-test).

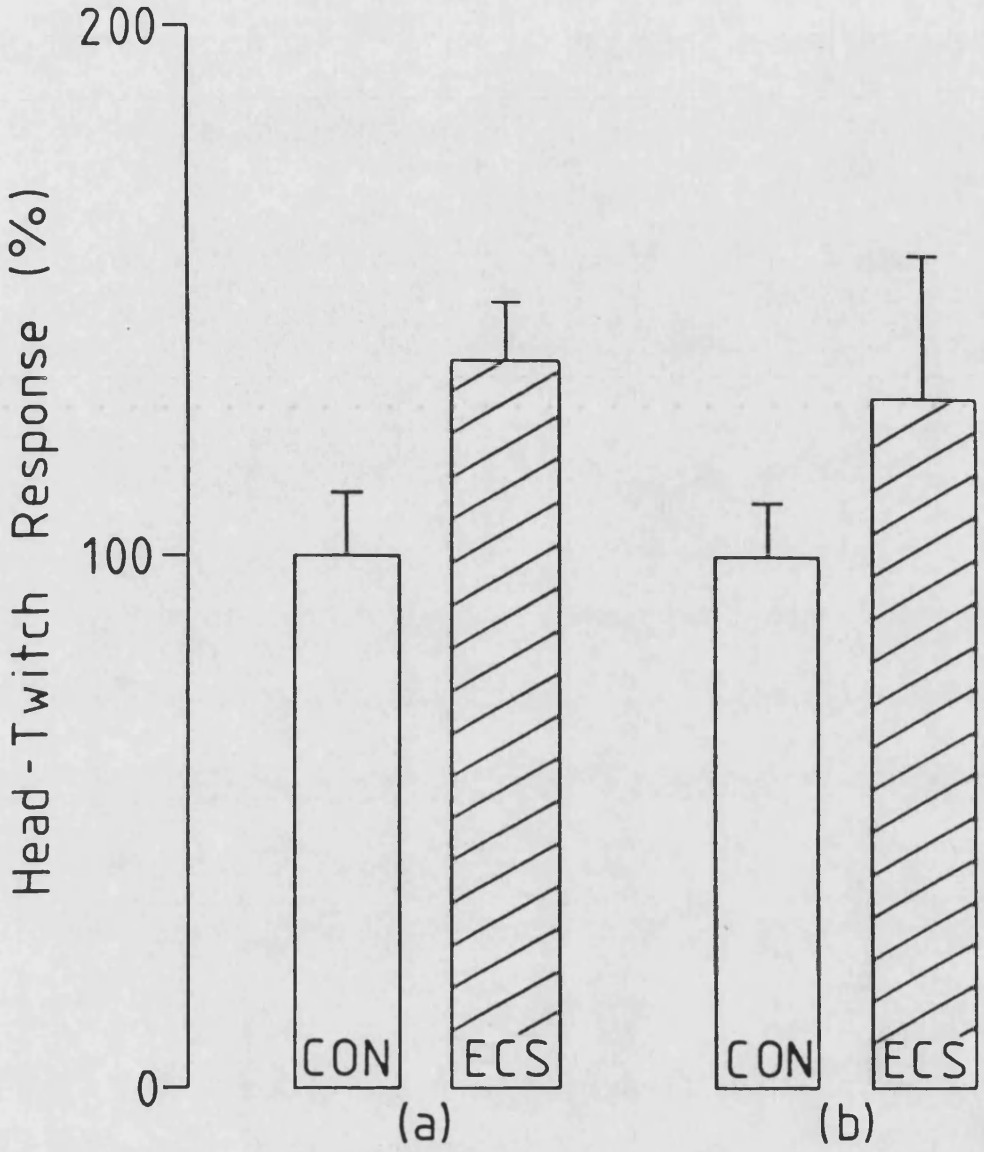


Figure 10.2 Mean head-twitch response in mice, 3 hours after a single ECS, evoked by (a) 5-MeODMT (5 mg/kg, i.p.) or (b) 5-HTP (100 mg/kg, i.p.).

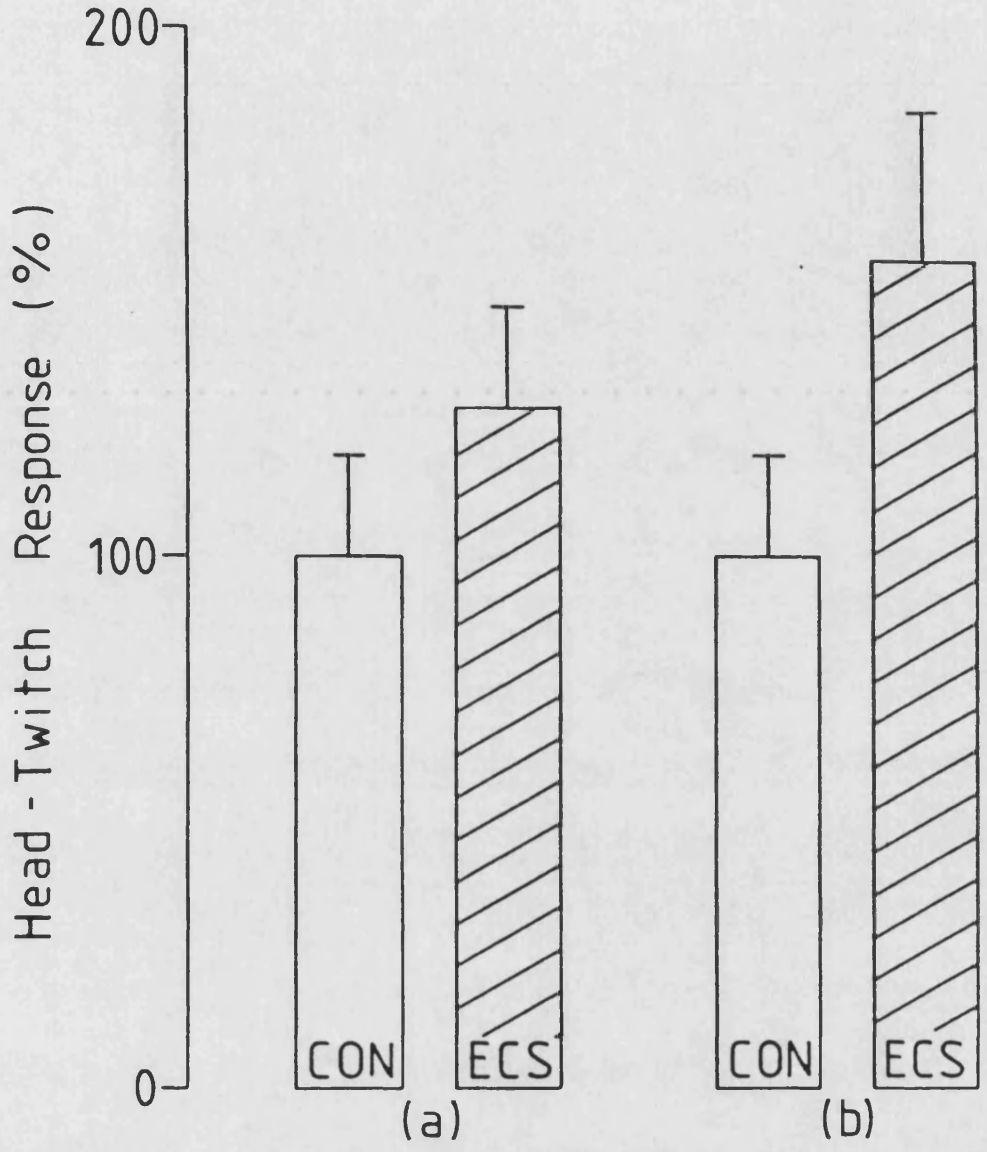


Figure 10.3 Mean head-twitch response in mice 24 hours after a single ECS, evoked by (a) 5-MeODMT (5 mg/kg, i.p.) or (b) 5-HTP (100 mg/kg,i.p.).

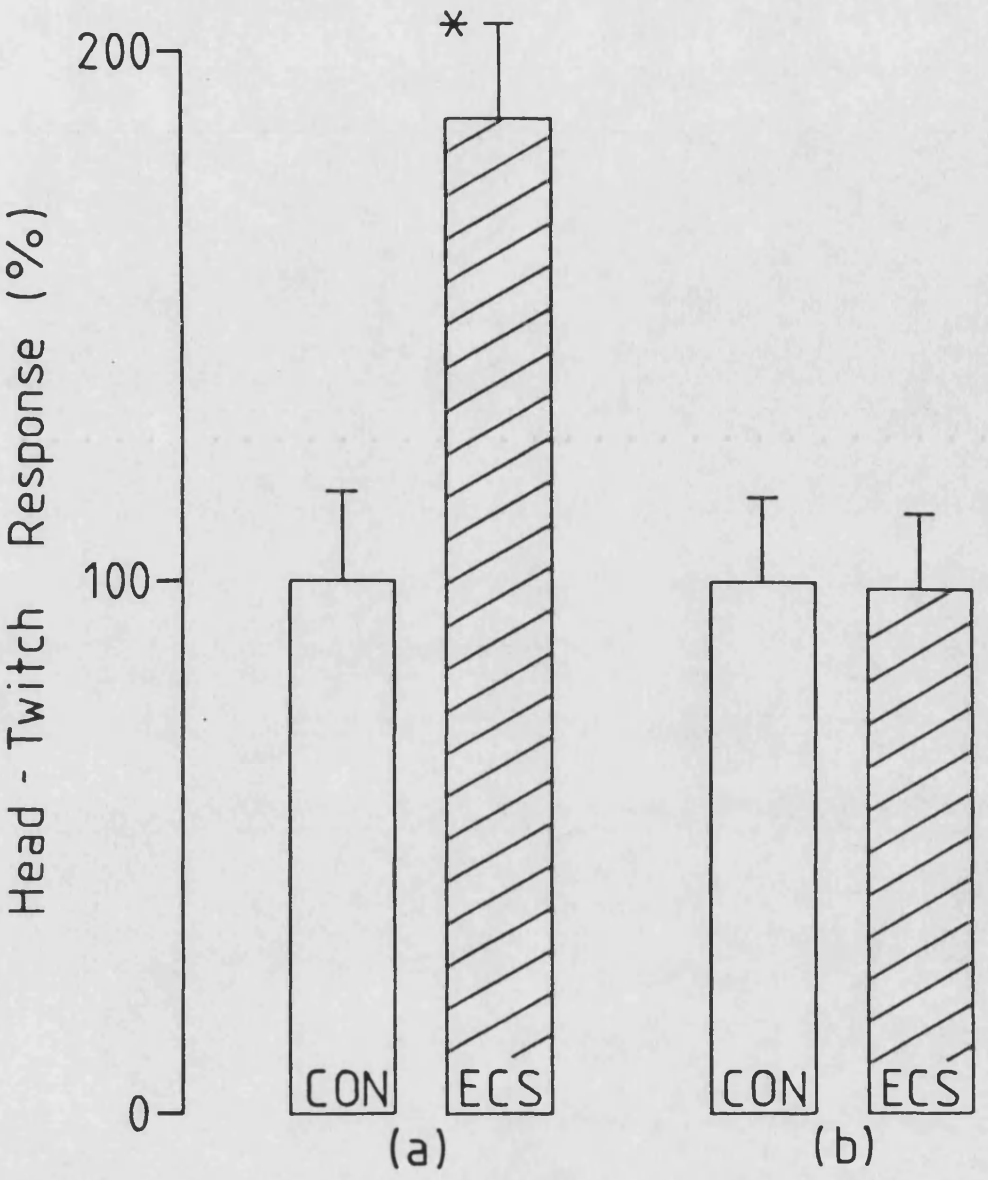


Figure 10.4 Mean head-twitch response in mice 48 hour after 7 x ECS, evoked by (a) 5-MeODMT (5mg/kg, i.p.) or (b) 5-HTP (100 mg/kg, i.p.). *: $p<0.05$ (t-test).

10.3. Results

Chronic consumption of mianserin for 18 days decreased the mean head-twitch response to a level significantly lower than control ($p<0.01$), an effect which persisted 48 hours after drug withdrawal (Fig. 10.1). Administration of DMI produced only a slight, non-significant decrease in the response on the 18th day of drug administration, returning to levels identical to control 48 hours after drug withdrawal (Fig. 10.1).

The use of the higher dose of 100mg/kg 5-HTP had the same outcome (results not shown).

Compared to chronic antidepressant drugs, ECS produced the opposite effect. Acute treatment enhanced the head-twitch response evoked by either 5-HTP or 5-MeODMT, though not to a significant extent (Figs. 10.2 and 10.3). Chronic ECS almost doubled the response observed after 5-MeODMT but did not alter the response following 5-HTP (fig. 10.4).

10.4. Discussion

This series of experiments is essentially based on a number of experiments described by Goodwin et al (1984). The use of a single ECS and the evocation of head-twitches by both 5-HTP and 5-MeODMT complement the use of two antidepressant drugs and chronic ECS.

It is very difficult to standardize the administration of ECS without the use of EEG and the utilization of observation of chronic and tonic seizures as a proof of successful ECS administration can be subjective. One objective of the experiments discussed here was to provide evidence that the efficacy of ECS, as used in this laboratory, is comparable to that of other workers.

The head-twitch response is thought to be mediated by 5-HT_2 receptors in the mouse brain (Goodwin and Green, 1985; Goodwin et al, 1984; Green, O'Shaugnessy, Hammond, Schachter and Grahame-Smith, 1983c). It has also been shown to exhibit a circadian variation in response to 5-MeODMT (Moser and Redfern, 1985). The latter causes its effects by direct stimulation of 5-HT_2 receptors, whilst 5-HTP, following carbidopa-induced peripheral inhibition of aromatic L-amino acid decarboxylase, induces an increase in synthesis and availability of 5-HT for synaptic transmission.

The results show that chronic administration of mianserin decreases the head-twitch response, an effect only marginally reversed when the drug was withdrawn from the drinking water. These results are in agreement with those of Goodwin et al (1984) and Metz and Heal (1986). Unlike the results of the former, however, DMI did not cause an equally significant decrease in the response after chronic treatment, although the trend was present, nor did it precipitate an increase upon withdrawal; in the present study the response returned to control levels. Similarly, Metz and Heal (1986) found an enhanced response to 2 mg/kg 5-MeODMT after 1,2 or 3 ECS. Also, Goodwin et al (1984) reported a significant increase in the response to 5-HTP following repeated ECS. These findings are in partial contrast with the present results: acute ECS showed a non-significant enhancement of the response to either 5-HTP or 5-MeODMT, 3 and 24 hours after treatment. At 48 hours after repeated ECS, an increased response was found only to 5-MeODMT but not 5-HTP.

There are a few differences between these studies which might explain, in part, the variance of the results: (a) Both Goodwin et

al (1984) and Metz and Heal (1986) used male mice as opposed to female mice used in the present study, whilst both groups used also different strains of animals; (b) Goodwin et al (1984) administered 5 ECS over 10 days compared to 7 ECS over 13 days in the present study. (c) This study used a 20-minute interval between injections of carbidopa and 5-HTP and between 5-HTP and observation compared to the 15-minute intervals of Goodwin et al (1984). (d) Metz and Heal (1986) observed the animals for 6 minutes, twice the time of observation in this study.

Notwithstanding the methodological disparities, the present results also seem to suggest that repeated ECS induces an enhancement in the 5-HT_2-mediated head-twitch response in mice following 5-MeODMT and a decrease in the same behavioural response after chronic antidepressant treatment. These findings are supported by the reports of up- and down-regulation of 5-HT_2 binding sites in the mouse cortex following chronic ECS and antidepressant drugs, respectively (Goodwin et al 1984; Metz and Heal, 1986) but not by earlier studies using lower doses of DMI and different injection-experimental time intervals (Green et al, 1983b).

The present results are not in complete agreement with those in the literature. Nevertheless, it is considered that they provide sufficient evidence in support of the initial hypothesis that ECS was administered in an effective way. The fact that 5-MeODMT, but not 5-HTP, enhanced the head-twitch response following repeated ECS might be indicative of increased MAO activity. Then, even though ECS would apparently increase the number of 5-HT_2 receptors available for stimulation by 5-MeODMT, the increased availability of 5-HT following carbidopa and 5-HTP would not produce the same effect because of

excessive activity of MAO.

It has been shown in the past that MAO activity per milligram protein in rat brain increases slowly after repeated ECS, although enzyme concentration is basically unaltered (Pryor, 1974). However, these changes were detectable after at least one week of daily ECS treatment and more pronounced when the number of treatments increased to 15 to 30, at which point the changes persisted long after treatment was stopped (Essman, 1978b; Fink, 1979; Pryor, 1974). Considering also that repeated ECS was not found to alter 5-HT turnover (Modigh, 1976), an increase in MAO activity could lead to decreased 5-HT levels and a consequent receptor up-regulation, which would enhance, as it did, the response to 5-MeODMT but not 5-HTP. However, judging from the findings in rat CSF, no such increase of MAO activity can be surmised under the present conditions of ECS administration.

CHAPTER 11. DISCUSSION

11. Discussion

The work presented here had one main target: to examine the possibility that the antidepressant efficacy of ECT in clinical practice might be related to an ability to modulate circadian rhythmicity.

Before attempting to evaluate the relevance of the present findings in the broad context of current ideas about depression, 5-HT, circadian rhythms and ECT, it might be useful to recall the objectives of the four sets of experiments, to summarise the major conclusions drawn from them and to speculate on possible implications. The following six areas will be discussed.

(a) tryptophan and 5-HIAA concentrations in rat CSF measured over 24 hours;

(b) the effects of ECS on CSF concentrations of tryptophan and 5-HIAA;

(c) the enhancement of the head-twitch response following ECS;

(d) locomotor activity and ECS;

(e) passive avoidance and ECS; and

(f) an overall conclusion on the effects of ECS.

The final pages will be devoted to suggestions for further research work.

(a) The first conclusion was that tryptophan and 5-HIAA concentrations exhibit a diurnal variation in rat CSF. What this finding implies is that there is a temporal variation in the amount of 5-HT that is converted to 5-HIAA. What this finding does <u>not</u> indicate, however, is whether this variation is a consequence of a variation in MAO activity, 5-HT synthesis or 5-HT release.

If MAO activity exhibits a diurnal rhythm, it would be tempting to link it with the rhythm in 5-HIAA concentration. This interpretation would require that tryptophan availability exceed, at any time point, the enzymatic capacity of MAO and that the levels of 5-HT required for the normal function of the neuron be only of minor importance in regulating 5-HT availability.

If MAO activity does not display a rhythm, the changes with time in 5-HIAA concentration might be linked to variation in tryptophan availability in the brain, which would be consonant with the present finding of diurnal variation in tryptophan concentration in CSF. However, there is evidence that tryptophan availability does not enhance 5-HT synthesis (Elks et al, 1979b).

Alternatively, the rhythm for 5-HIAA concentration could either be associated with a direct circadian rhythmicity of 5-HT release or be an effect of feedback regulation from a circadian rhythm of postsynaptic function. Since a rhythm for 5-HT receptor binding has been postulated in rat brain it would seem reasonable to expect that it would, in turn, influence the amount of 5-HT release by the neurons.

However, one has also to consider the evidence for the existence of a rhythm in 5-HT synthesis (section 3.6.3.); various steps along the metabolic pathway of 5-HT seem to exhibit a rhythm. If the net effect is a rhythmic production of 5-HT and we have already accepted a rhythm in postsynaptic function, could it be perhaps that the rhythm in 5-HT receptor numbers regulates synthesis of 5-HT? The first option, of an effect on 5-HT release, seems more plausible and also may explain the existence of two pools for 5-HT at the nerve ending.

It should not be assumed, on the basis of this speculation, that other relations cannot exist; for example, the relationship could be an inverse one and a rhythm in 5-HT synthesis or release might dictate the rhythm in postsynaptic receptor number or activity. A conclusive answer will not be found if we do not elucidate the primary circadian component that is regulated by a pacemaker; otherwise, 5-HT function will remain a conglomeration of diurnal rhythms for which no internal synchrony and interdependence will have been shown.

(b) The ineffectiveness of ECS to alter the resting levels of 5-HIAA and tryptophan concentration in CSF can be taken as an indication that it does not interfere with processes affecting tryptophan availability or 5-HT synthesis, release and metabolism. Moreover, ECS appears to be unable to affect the mechanisms that control the diurnal variation of these substances in CSF. The results do not support the view that ECT may interfere with circadian rhythmicity as part of its action.

(c) On the other hand, the results obtained from the head-twitch experiments serve to support the existing hypothesis that the therapeutic action of ECS may be mediated by changes in postsynaptic function. The failure of ECS to cause an enhancement in the head-twitch response evoked by 5-HTP in ECS-treated animals seems to support the idea that precursor availability does not necessarily influence 5-HT activity. Since there is a measurable response to 5-HT and carbidopa, it means that the drug treatment has, in fact, produced the expected result. The lack of enhancement of the response by repeated ECS implies that

this increase in 5-HT synthesis was insufficient to elicit a higher response or that it was counteracted by an opposing effect of ECS. Such an effect has not been accounted for, so far. Moreover, an effect of ECS on MAO activity has already been excluded since it would entail a decrease in 5-HIAA concentration in CSF, which would contradict the present findings.

In addition, it is known that ECS increases the number of 5-HT_2 receptors (e.g. Green et al, 1983b) and we have argued that postsynaptic receptor function may influence the concentration of 5-HIAA. On the other hand, the present results from the head-twitch response in mice also lead to the assumption that the number of 5-HT_2 receptors has increased, whereas the findings from rat CSF indicate that 5-HIAA levels are unaltered, following repeated ECS. It may thus be inferred that the regulation of the concentration of 5-HIAA is not dependent on the postsynaptic function of 5-HT, at $5HT_2$ receptors.

(d) The study on the effects of ECS on the locomotor activity rhythm in mice and rats has yielded no positive results. It could be concluded that the mechanisms underlying the expression of the rhythm are resistant to the application of ECS. It is worth pointing out that the resistance may be lowered by disease or even share in the pathogenesis of depression; under those conditions it might be easier to modify the rhythm.

An important question that has not been answered is whether ECS affects specific behaviours that contribute to the final activity rhythm. It may be that ECS influences, for example, grooming and exploratory behaviour, possibly in such a manner that the effects

cancel each other out. It is tempting to speculate that certain behaviours may be more vulnerable than others, but it is difficult to conceive how these changes almost invariably result in no overall alteration of the rhythm.

Nevertheless, the experiments provided the chance to examine the nature of a true circadian rhythm. Thus, the locomotor activity rhythm was entrainable to a L:D sequence and slowly freeran under constant light or dark, with a frequency presumably controlled by an endogenous pacemaker. The freerunning rhythm successfully re-entrained to L:D conditions. The slow transition between two L:D cycles and the establishment of a freerunning rhythm under constant conditions constitute two of the major criteria for the characterization of circadian rhythms.

Finally, an interesting species difference was found when rats and mice were allowed to freerun in constant dark. The results confirm the evidence reviewed by Bünning (1973).

(e) Both anterograde and retrograde amnesia were observed, under various circumstances, in mice treated with repeated ECS, a finding that supports the overwhelming clinical evidence for memory impairment after ECT. As already discussed, the response to the aversive stimulus in the test (footshock) may not be exclusively connected to learning and retrieval but, depending on the time of the day, may relate to variables like locomotor activity.

When the animals were exposed to ECS, a compounding factor could be that the treatment has affected locomotor activity. However, judging from the locomotor activity results, no such supportive evidence is offered. Still, a possible involvement of ECS should not

be dismissed so easily. It has been argued in Chapter 8 that there may be more than two components of locomotor activity (Hutchins and Rogers, 1973), one of serotonergic and one of catecholaminergic origin. Morever, Cools (1986) showed that a dopaminergic component of locomotor activity is not only dually regulated by two different brain areas (olfactory tubercle and medial nucleus accumbens) but is also dependent on time of day. In a test like the one under consideration, therefore, the possible indirect influence of locomotor activity should not be excluded.

Yet another implication is the effect of ECS on the opioid peptide system of the brain: an increase in endorphin release might overcome the noxious effect of contact during crossing from plate to plate, leading to a false assumption of memory impairment, when what actually happens is a neutralization of the noxious stimulus.

In order to investigate this point, the hot-plate test for analgesia was used with control and ECS-treated animals (results not shown). No indication was found of an enhanced analgesic response of the treated versus untreated animals, but it must be conceded that the test is not specific for endorphin function.

(f) The conclusion to be drawn from all the points discussed so far is that ECS does not appear to interfere with circadian organization. This does not mean that ECS cannot be ultimately shown to affect circadian rhythms; it may do so indirectly by rectifying the damage of a system that is implicated in the generation or maintenance of rhythmicity and functions abnormally in depression. Inevitably, this hypothesis cannot be tested on, presumably healthy, experimental

animals. On the other hand, whenever an effect was attributed to ECS, chronic, rather than acute, administration of the treatment was responsible. This is in agreement with the vast majority of the experimental and, in part, the clinical evidence in the literature; it is also an important common characteristic between ECS and ECT (Grahame-Smith et al, 1978).

It is, perhaps, appropriate at this point to remark that the literature contains a disappointing variety of conditions of ECS administration: the voltage varies between 120 and 150V, the duration of the stimulus between 0.2 and 2 seconds, the current between 17 and 150 mA, the number of treatments between 3 and 10 and their frequency between one daily and three weekly ECS. With such a variation on top of other confounding factors like the use of different animal strains and analysis techniques, it is inevitable to expect discrepancies in the reported literature.

There is not much to add, by way of putting forward possible explanations of the results. The reader is now invited to examine a few observations that evolved from the review of the existing literature and are concerned with more general ideas about depression and 5-HT, beyond the direct relation with the results.

The first topic deals with the pathogenesis of depression. If the symptoms of depression are examined carefully, it becomes apparent that the illness manifests itself in a variety of ways. Mood and sleep disturbances are prevailing as are abnormalities in appetite and libido and a multitude of other somatic manifestations of the illness that resemble the symptomatology of anxiety neurosis. Thus it is not unreasonable to assume that the disturbances are due to abnormal function of numerous neurotransmitter and hormonal systems.

Also, it is well accepted that most behavioural and physiological events are usually the expression of more than one system and furthermore each system, whether neuronal or hormonal, is commonly affected by the function of other systems.

In addition, it should be remembered that as a response to malfunction of a system, the body may invoke compensatory mechanisms in the same system or a hyper- or hypo-activity, as the case might be, in other systems, in order to counteract the abnormality. Consequently, it could well be expected that the form of the illness may vary dramatically from patient to patient, depending on the degree of the original abnormality and the compensatory processes. If this view is correct, depression may represent either the initial failure of one (or more) system(s) or the failure of both the system(s) and the compensatory mechanisms. Since, according to this concept, the illness may take many forms, it can be expected that clinical or laboratory findings from patients cannot always be used to classify them into groups.

Also, since we do not know the causes of the illness and we cannot follow its course from the beginning, the examination of patients inevitably provides an indication of their state, but not a guide to the actual stage of the illness. As a consequence of all these, it may be postulated that a certain treatment will have a varying degree of success among patients. Conversely, patients might require different treatment according to the prevalence and degree of their symptoms. This brings the discussion to the second topic.

The clinically useful antidepressant treatments in current psychiatric practice include ECT, tricyclic antidepressant drugs

(TCAs), atypical antidepressant drugs, MAO inhibitors and also lithium for the prevention of recurrence of depressive episodes. If the treatment of choice fails, another one will be tried and so on until a therapeutic effect is accomplished.

On the other hand, one cannot help wondering at this remarkable diversity of effective treatments and is compelled to attempt to locate a possible common component that all the treatments may share. This would essentially reflect a common basic abnormality in all patients. In this effort, much is to be learnt from the putative mode of action of each treatment; even more might be gained from their side effects.

As discussed in the introduction, the principal mechanism that has been identified and considered as the therapeutic ingredient for MAO inhibitors is the inhibition of MAO and a consequent increased availability of neurotransmitter. For tricyclic antidepressant drugs, the main element is inhibition of neurotransmitter uptake and for atypical antidepressants possibly an effect on receptor level. ECT is thought to enhance postsynaptic response to NA, 5-HT and dopamine. As for lithium its mode of action is more obscure and may involve regulation or readjustment of neurotransmitter release by action on the presynaptic membrane; stabilization of conformational states of receptor sites; influence on membrane ATPase and hence transmitter release and on the membrane sodium pump; effects on postsynaptic cAMP system.

The antidepressant drugs, in a way, have more limited but more selective targets than does ECT. Due to their physicochemical properties, they accumulate in the brain, among other tissues, and their side effects are extensive and mainly due to action similar to

their therapeutic mechanism.

ECT is not only localized, as far as application area is concerned, but it also reaches brain structures to varying extent, depending on their location. The very nature of the treatment almost instinctively dictates that its target is also electrical in nature. Effects on neurotransmitter turnover and even receptor numbers are the essence of the treatment; the core must be the neuronal membrane. ECT (and ECS) may affect the conductivity of the neurons, primarily, and also the permeability of the neuronal membrane to ions and possibly transmitter precursors. Moreover, the ionic movements could affect the conformational state of receptors and possibly the adenylate cyclase and phosphatidyl inositol second messenger systems. Since the treatment is applied for only seconds every two or three days, the effects, which must be additive, since they depend on the number of treatments, wear out at some point after discontinuation. If the targets of ECT are the ones postulated above, it is not surprising that lithium, with its stabilizing properties, maintains the remitted state and prevents relapse.

Ultimate failure of lithium may simply reflect our failure to monitor adaptive changes (or return to normal state) of the diseased organism to the new steady state,and to reconsider the therapeutic approach.

It is worth examining, in the third topic, possible therapeutic targets, for which evidence has accumulated from the application of all or most of the useful antidepressant treatments. Sulser et al (1978) studied the evidence for the effects of antidepressant treatments on the noradrenaline (NA)-sensitive adenylate cyclase

system, in rat limbic forebrain. Chronic treatment with tricyclic antidepressants, MAO inhibitors, iprindole and ECS shared the ability to reduce the cAMP response to noradrenaline. Evidence has also been provided for lithium, inhibiting NA-sensitive adenylate cyclase in rat cortex (Knapp, 1983).

According to Sulser et al (1978), depression is "a reflection of a state of pathological hypersensitivity of catecholaminergic receptors in brain" and their proposed model for the illness and its alleviation manages to incorporate a large part of the available data on NA-system.

A major problem with the proposals of Sulser et al (1978) was the fact that they did not account for disturbances of any other system; yet, there is an enormous amount of data which reveals that at least three major system (5-HT, dopamine and noradrenaline systems) are involved in the pathophysiology of depression either due to altered transmitter availability or receptor hypersensitivity and that these systems are, functionally, closely related to each other (Curzon, 1982; Eccleston, 1981).

In particular, the 5-HT system cannot be easily ignored. Apart from biochemical evidence suggesting a role for it in the genesis or expresion of depression (Curzon, 1982; Lapin and Oxenkrug, 1969; Shopsin et al, 1976), many of the major symptoms of the illness have, in some fashion, a serotonergic component: sleep, mood, appetite, libido. It has, therefore, attracted a lot of interest in research. For the sake of comparison, in a similar manner, dopamine malfunction may account for the motor retardation observed during the illness (van Praag, 1981).

Although so much evidence is considered to support the

hypothesis of abnormal 5-HT and possibly NA and DA function, it should not be concluded that no other systems are involved. The importance of peripheral functions should also not be underestimated and possibly deserve more attention. Similarly, the role of secondary metabolites of the major neurotransmitters should also be considered.

One of the systems that needs careful examination is the system of endogenous opioid peptides, since there is evidence supporting a role for endorphins and enkephalins in the regulation of affect and the pathophysiology of depressive illness (Barchas, Madden, Weber, Evans and Berger, 1983).

Interestingly, it has been reported that β-endorphin displayed a diurnal variation in both plasma and CSF of hydrocephalic patients, with normal blood-brain barrier (Barreca, Siani, Franceschini, Francaviglia, Messina, Perria and Rolandi, 1986) and in plasma of controls (Gil-Ad, Dickerman, Amdursky and Laron, 1986).

The opioid peptide systems is fairly responsive to antidepressant treatment. ECT caused an increase in plasma β-endorphin immunoreactivity in depressed patient but the effect which was still significant at 20' post-treatment fell to pretreatment levels within 48 hours (Misiaszek, Cork, Hammeroff, Finley and Weiss, 1984). Whilst repeated ECS did not affect β-endorphin levels in brain, it increased met-enkephalin concentration in selected rat brain areas (Hong, Gillin, Yang and Costa, 1979). Finally, chronic DMI or ECS administration did not affect the number of opioid receptors in rat forebrain although lithium has been shown in the past to down-regulate them.

Another target that could be implicated in the pathogenesis of depression and would be expected to be influenced at least by ECT and lithium are the concentrations of different ions, particularly calcium, in the brain. There is long-standing, incontrovertible evidence for the role of calcium in the conductance of nerve impulse, neurotransmitter release, enzyme activity and second messenger systems. Using rat brain slices, Elks, Youngblood and Kizer (1979a) provided convincing evidence that both synthesis and release of 5-HT are dependent on the flux of calcium ions through the neuronal membrane. Lithium, which increases intracellular calcium and reduces the resting membrane potential could block the release but accelerate the synthesis rate of 5-HT following electrical depolarization, unless calcium was eliminated (Elks et al, 1979a).

These data show that calcium is actively involved in the synthesis of one neurotransmitter and that lithium can modify the interaction. Of course, much more data is required in order to show that calcium concentrations affect the metabolism and release of other neurotransmitters.

The determination of calcium in CSF has not yielded any particularly encouraging results. Following three daily ECS, an injection of labelled calcium resulted in increased entry of calcium into the CSF of rats without affecting the elimination rate. This result was attributed to increased blood-brain permeability (Barkai, 1983). On the contrary, in a study of depressed patients, CSF calcium concentration was determined in plasma and CSF prior to a course of ECT and two weeks after discontinuation of treatment. Calcium levels were significantly decreased in recovered patients but there was no sign of correlation between the levels in the two

body fluids (Carman, Post, Goodwin and Bunney , 1977).

Whilst there is conflict as regards the effects of calcium concentrations in CSF after ECT or ECS, there is also some doubt as to the relation between calcium levels and depression. Gerner et al (1984) found no difference in CSF calcium levels between normal controls and depressive patients. There was also no difference in a study of depressive and schizophrenic patients compared to neurological controls (Banki, Vojnik, Papp, Ball and Arató, 1985), despite an upward trend. However, these authors reported a significant correlation of magnesium ions with 5-HIAA concentration and also elevated magnesium concentration in a suicidal group from the same patients. It has been argued that magnesium may affect 5-HT neurotransmission, an action possibly enhanced by various antidepressant therapies (Banki et al, 1985).

Since both calcium and magnesium are omnipresent and involved in a large number of functions it would be unrealistic to expect that CSF levels of either of them would disclose with accuracy the location from which a decrease or increase evolved. Of course, the difficulties in interpreting findings from CSF in general are also apparent in this case. It should not be expected that any one of these cations would ever be "incriminated" in the causes of depression; altered levels of calcium or magnesium could be a consequence of a damage in a particular system rather than a cause.

The list of possible causes of depression and probable therapies could easily go on, but it would not offer anything constructive. The important message is that the illness is a complex pathological state and benefits from treatments that correct possibly

only parts of the abnormal responses. The 5-HT system appears to be one of the involved system in the illness and ECT one of the effective therapies.

Before we move onto the final piece of this thesis, which will offer a few suggestions for further research work, a last attempt to identify the importance of circadian rhythms in depression will be made.

One of the fairly characteristic symptoms of depression was said to be the diurnal mood swing. Some patients present a worsening in the morning and improvement towards the evening whereas some patients present the opposite picture. Fink (1979) clearly suggested that the latter group should be excluded from ECT treatment. This is presumably an advice based on clinical experience, but it strongly implies that there is a distinct phase in a patient's rhythm that could be more susceptible to ECT. This point justifies the search for a vulnerable phase of the locomotor activity rhythm and possibly even shows why it is so easy to miss.

For the last time succumbing to the temptation to speculate about the nature of these observations, it is worth mentioning that, apart from all the steps in 5-HT function that have been shown to display a diurnal variation, 5-HT neurons in rat SCN, thalamus, cortex and hippocampus display a circadian variation in their sensitivity to iontophoresed 5-HT (Mason, 1986). The variation appears to be a true circadian rhythm since it persists in freerunning conditions.

If this variation is generated in the SCN or any other pacemaker and is responsible for all the variations along the 5-HT pathway and ultimately in all 5-HT responses, it is evident that a loss of

synchronization will disrupt a significant part of the brain circuitry. Furthermore, if the released neurotransmitter can stimulate the 5-HT receptors which, it may be postulated, could exist in either of two conformational states, regulated by the ionic balance of the postsynaptic membrane and much along the lines proposed by Bunney et al (1977), alternate between two different states of sensitivity, the action of ECS and lithium would be easier explained. Antidepressant drug treatment could also fit in the same pattern and even allow for the delay in onset of therapeutic action (Blier and Montigny, 1985). This, however, is a highly speculative theory which perhaps poses more quesions rather than provides answers.

Suggestions for further work

There are numerous studies that could be undertaken, starting from the present results. Some of them would aim to produce more data using the same methods and some would set out to explore completely novel areas. A few suggestions have already been provided in the previous discussions.

A model for sampling rat CSF should be used to show whether the diurnal variation is a true circadian rhythm. The model should be reliable for sampling CSF from the same animals at hourly intervals and would probably be more suitable with larger animals. The animals would then be allowed to freerun in constant light (or dark) in order to investigate whether the postulated rhythm will persist and freerun, possibly, but not necessarily with the same frequency as the locomotor activity rhythm, which should also be monitored. It would

be equally interesting to establish the same variation reported here in probenecid-treated animals.

If a true circadian rhythm for tryptophan or 5-HIAA concentration in CSF emerges, it should then be subjected to chronic administration of antidepressant drugs or ECT, again under freerunning conditions in order to investigate the susceptibility of the rhythm. This might provide data as to whether antidepressant therapies act indeed by interference with the circadian pacemakers.

Manipulations, like food-deprivation, and drug treatments which had an effect on tryptophan and 5-HIAA concentration in CSF should be applied to animals tested simultaneously for 5-HT receptor function with or without ECS treatment. These experiments would provide the measurement of variables over 24 hours whilst evaluating both 5-HT synthesis and postsynaptic function.

It is of particular interest to examine the effects of combined ECS and antidepressant drug treatments on receptor binding, not necessarily limited to 5-HT receptors. The rationale is to examine the impact that the two therapies would have on receptor numbers, since it is known both that they occasionally have opposite results and that, in clinical practice, they may be administered together.

The experimental design I have employed to measure locomotor activity may be significantly improved by simultaneous monitoring of feeding and drinking behaviours, social interactions and temperature. Such a setup is feasible and would possibly help to identify a rhythm or a group of rhythms (and, therefore, possibly a pacemaker) that may respond to antidepressant treatments.

An interesting project would be the conversion of the

environmental cabinets in such a way that the rats can regulate their own light-dark cycle. This type of experiment is not original since it has been applied at least in birds, with limited success. In theory, the experiment should provide ultimate conditions for establishing a freerunning rhythm.

It has been shown in the past that certain methylxanthines influence the rest-activity cycle (Bünning, 1973; Engelmann et al, 1983). In view of the widespread consumption, even among patients, of beverages and soft drinks which contain methylxanthines, the possibility that these substances can affect circadian rhythmicity seems to be worth examining.

Apart from the lack of effective animal models of depression and the obvious handicap of administering drug treatments or ECT to, presumably, normal (healthy) organisms and drawing conclusion from them about the action of the same treatments on depressed patients, we do not have a model to imitate the circadian changes in depression. Thus, a final kind of experiment on locomotor activity would be the challenging task of producing and maintaining animals in a phase-advanced rhythm. Up to now, this target has not been reached.

It has already been mentioned in section 9.4 that more experiments are required in order to elucidate the amnesic effect of ECS and ECT. Changes in the voltage, frequency and duration of the stimulus should vary as a means of determining a combination that can minimize or dispose of unwanted effects on cognitive functions. Two requirements are imperative in this direction. The first is EEG monitoring of the experimental animals, so that there is an objective and valid measure of brain seizure. The second is the use of another

model for determining memory function. The new model should be a positive reinforcement model. The objective is to identify possible varying effects of ECT on mechanisms mediating the learning and retention of avoidance responses and responses which may affect the survival of the animal. In conjunction with drug treatments that specifically affect the serotonergic, cholinergic or other systems, the experiments might contribute to our knowledge of the biochemical and anatomical foundation of cognition.

On a clinical level, there are certainly many directions that one can follow but the experimental background of this thesis does not provide the right information to stimulate suggestions for clinical research. Still, there are two intriguing aspects of therapeutics that have shown encouraging results and fit in the pattern of circadian abnormalities in depression: sleep- and light-therapies. Both types of therapy have a sound scientific base, if one accepts the concept of disrupted circadian rhythmicity in depression, and have the advantage of being relatively safe to apply.

Finally, there is a proposal for a study on a clinical level, which must have been frequently considered and never initiated, since it would require enormous organization. There are indications that affective disorders may be inheritable but a genetic marker has, in vain, been sought. A study should, therefore, be conducted on first degree relatives of patients with an established form of the illness. The study would follow up the subjects from an early age until one generation later with frequent assessment of both biochemical and clinical measures, in the hope that the appearance of the illness will correlate with,or even be predicted from, changes in those

measures. The enormity of the task, in terms of human and financial resources, and the necessary response from the required individuals negates the hope of it being materialized.

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